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Acute Atrophy of Bone (Osteoporosis) Simulating Hyperparathyroidism[1,2]

[Osteoporosis]

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TO DISTINGUISH between osteitis fibrosa generalisata resulting from hyperparathyroidism and osteoporosis should, as a rule, be simple (1, 2). Under one particular set of circumstances, however, the two conditions may closely simulate each other. It is the purpose of this paper to report one case in which an erroneous diagnosis was arrived at because the authors were not aware of certain points which this case illustrates.

To avoid confusion as to terminology the following rather dogmatic classification of metabolic bone conditions associated with demineralization is here inserted. The structure of bone tissue is constantly undergoing change. There are surfaces where bone is being resorbed and where one sees osteoclasts; there are other surfaces where the organic matrix is being laid down by the osteoblasts and where calcium salts are being deposited into it from the tissue fluids. A diminution in the bony mass may result from either an increase of bone resorption or a decrease of bone formation. The former condition is termed osteitis fibrosa generalisata and is met with in hyperparathyroidism; the latter must be further sub-divided. Thus a decrease of bone formation may result either from an underactivity of the osteoblasts in laying down the organic matrix or from a failure in the deposition of the inorganic calcium salts into the organic matrix; the former condition is termed osteoporosis, the latter osteomalacia.

There is considerable circumstantial evidence that the stimulus for the osteoblasts to lay down new organic matrix is stresses and strain. In the absence of these as when a limb is immobilized in a plaster cast there results a loss of bone mass, it is believed, because bone resorption continues and new bone formation decreases. Atrophy of disuse, therefore, is a special form of osteoporosis.

Since it is probable that osteoporosis is primarily a disorder of tissue metabolism and only secondarily

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one of calcium metabolism one is not surprised to find in this condition that the serum calcium and phosphorus values are usually normal. Furthermore, since the phosphatase level of the serum is presumably an index of osteoblastic activity one would not expect it to be elevated; if anything one would expect the opposite. As a matter of fact the level in osteoporosis

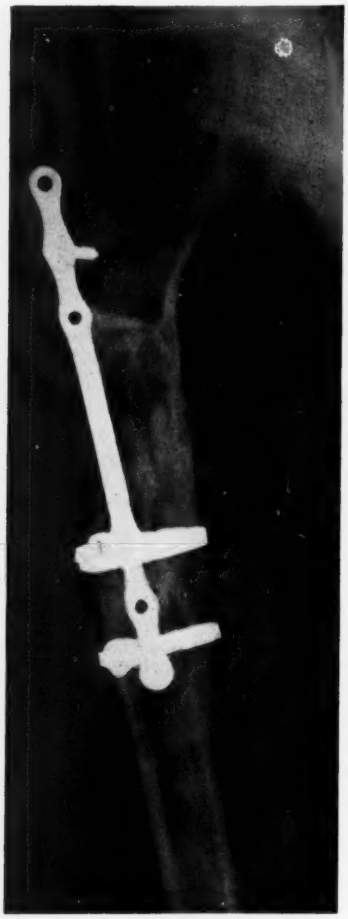

Fig. 1. X-RAY SHOWING CYST AND FRACTURE IN FEMUR AFTER OPEN REDUCTION.

is usually normal. However, since bone resorption continues unabated and bone formation is decreased, it follows that hypercalcinuria will be present in osteoporosis just as it is in hyperparathyroidism. Furthermore, it may lead to the same kidney complications. Thus, in contrast to the hypercalcinemia, the hypophosphatemia, and the hyperphosphatasemia in osteitis fibrosa generalisata due to hyperparathyroidism, one expects to find in osteoporosis normal values for these three variables. In this paper one important exception to this statement will be pointed out.

CASE HISTORY

The patient (*M.G.H. 260916*), a 14-year-old schoolboy, seemed perfectly well and active until two months before his admission on July 26, 1940 when, in a baseball game, he slid into home plate after making a home run and fractured the neck of the right femur. An x-ray (fig. 1) demonstrated a cyst at the site of the fracture but normal bones elsewhere. He underwent an open reduction and was put into a spica cast which immobilized him from the waist down. It is of interest in view of the later findings that a urine examination at this time showed nothing abnormal and that the specific gravity of the urine was 1.024. Curettings from the bone cyst showed osteitis fibrosa on histological examination.

The patient did not do well and suffered from anorexia and episodes of vomiting. He developed hematuria on one occasion but no gravel was demonstrated. The surgeon in charge, Dr. Alexander P. Aitken, found a hypercalcinemia and, very logically, concluded that the patient had an underlying hyperparathyroidism as the cause of his cyst, and that the gastric symptoms were the result of the hypercalcemia per se. He was referred to the Massachusetts General Hospital for further study.

On admission the patient had a high serum calcium level, 14.6 mg. per 100 cc., a normal serum phosphorus level for a growing boy, 4.5 mg. per 100 cc., and a normal serum phosphatase level for a growing boy, 6.0 Bodansky units. There was extreme decalcification of the immobilized parts of his skeleton but not of the remainder of his skeleton (fig. 2A, 2B). The lamina dura was easily visible (fig. 3). The latter consists of a lamina of bone in the jaws bordering the tooth sockets. Its visibility by x-ray is strong evidence against a generalized decalcification due to hyperparathyroidism (3); on the other hand, in most forms of osteoporosis it is unaffected.

The urine contained albumin and large amounts of calcium as shown by rough tests, and the sediment showed many calcium phosphate casts (4). In three urine concentration tests he was unable to concentrate above 1.012. Intravenous pyelogram on two occasions showed no filling of the left kidney pelvis. On the other hand the phenolsulphonephthalein excretion was almost within normal limits and the urine reached a pH of 5.5 on occasions.

Differential Diagnosis. The bone cyst and the hypercalcinemia, of course, suggested hyperparathyroidism. There were, to be sure, several considerations which were against this diagnosis. The serum phosphorus level was not depressed. However, in the presence of beginning kidney insufficiency, for which

there was considerable evidence, one can have hyperparathyroidism without hypophosphatemia. An aspect more difficult to harmonize with the diagnosis of hyperparathyroidism was the absence of generalized decalcification. One, of course, meets many cases of hyperparathyroidism without any evidence of bone disease (5) but one should not have a cyst due to hyperparathyroidism without generalized decalcification elsewhere. It seemed possible, however, that there was an underlying generalized decalcification so slight that it was not clinically demonstrable. Another possibility, of course, was that the patient had two diagnoses: *a*) hyperparathyroidism, and *b*) an unrelated solitary bone cyst. In the absence of a better diagnosis the patient was considered to have hyperparathyroidism. In view of the renal impairment already present, immediate operation was decided upon.

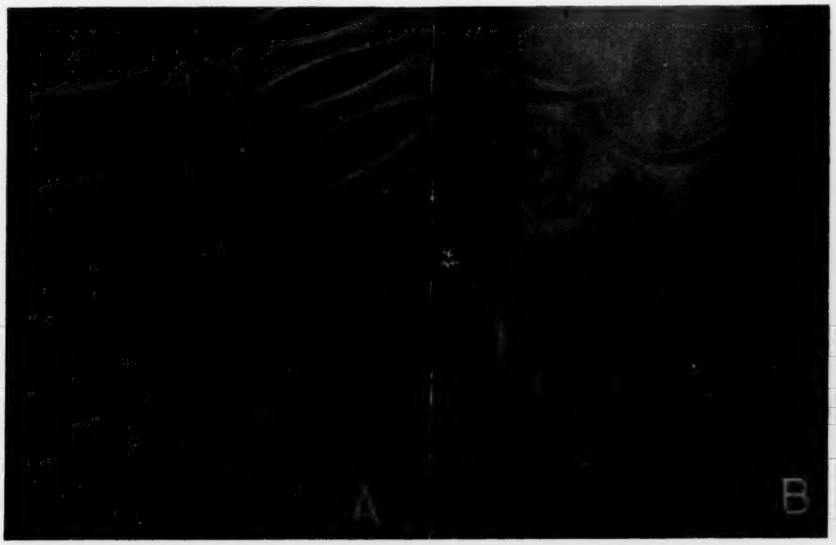

Fig. 2. X-RAYS CONTRASTING NORMAL DENSITY OF PARTS OF SKELETON NOT IMMOBILIZED (*hands*, A) WITH DECREASED DENSITY OF PARTS OF SKELETON IMMOBILIZED (*foot*, B).

Parathyroid explorations. At the first operation the entire neck region, posterior mediastinum and the visible portion of the superior mediastinum were explored (6). Three asymmetrically placed normal parathyroid glands were identified and specimens removed for histological examination from each. The lower gland on the right side was within the capsule of a tongue of thymus tissue which rose out of the anterior mediastinum. A longer tongue on the left side was excised but no parathyroid tissue was found. Since more thymus tissue obviously existed down in the chest it was thought that the tumor lay probably in the anterior mediastinum. A specimen was taken from the left tibia for biopsy. Histological examination of the glandular tissue showed three normal parathyroid glands, normal thymus tissue and normal bone.

Although uncertainty existed regarding the diagnosis, the second operation was undertaken approximately six weeks later. At this operation the sternum was split and the anterior mediastinum explored. The

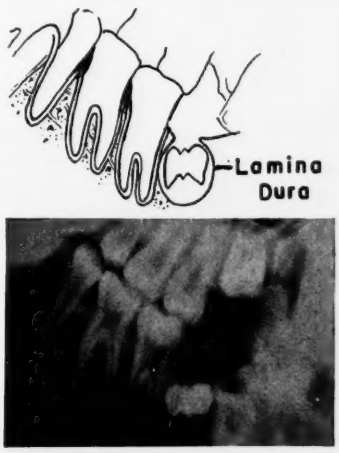

Fig. 3. X-RAY SHOWING PRESENCE OF NORMAL LAMINA DURA.

remainder of the thymus tissue was removed but no parathyroid tissue was found.

Denouément. When the exploratory operations failed to uncover a parathyroid adenoma the diagnosis of hyperparathyroidism was abandoned. It was decided that the following sequence of events offered the most plausible explanation: *a*) solitary bone cyst in an otherwise healthy, young boy; *b*) fracture through cyst; *c*) immobilization of a large part of a dansky units. With the reduction in the hypercalcinemia the anorexia and the vomiting disappeared, the calcium phosphate casts disappeared from the urine, and the patient once again could concentrate his urine satisfactorily. An intravenous pyelogram showed normal function on both sides, and the patient made an uneventful recovery. The course of the disease as shown by the blood chemical determinations is shown in figure 4.

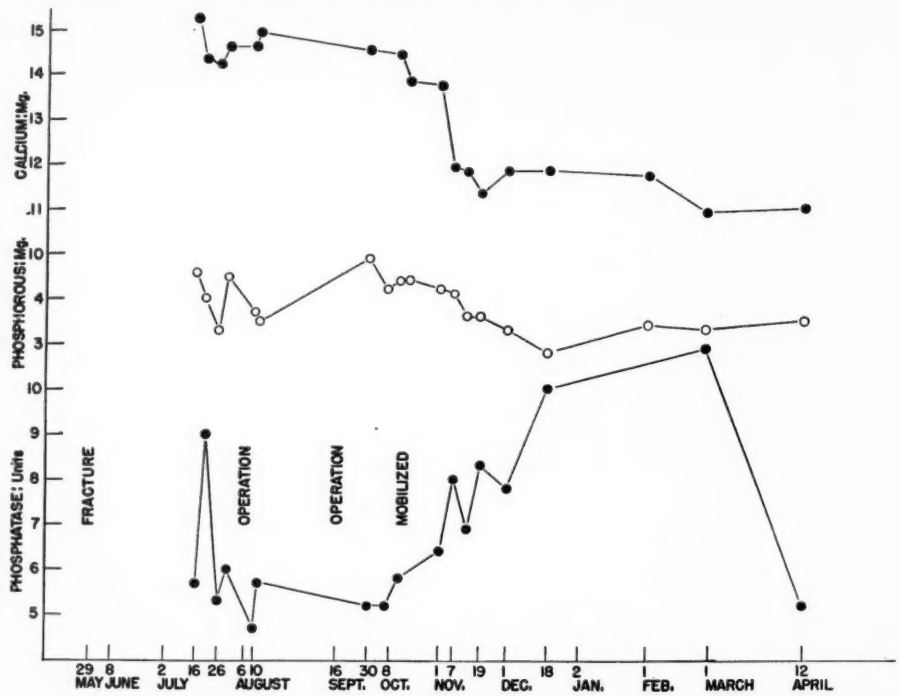

Fig. 4. Chart showing relations of serum calcium, serum phosphorus and serum phosphatase to time of the fracture, parathyroid explorations, and mobilization.

previously active skeleton; *d*) osteoporosis of disuse; *e*) an excess of calcium from the immobilized bone being presented to the kidneys for excretion; *f*) an inability of the kidneys to excrete promptly all the calcium; *g*) resulting hypercalcinemia; and, *h*) kidney damage from excess of calcium being excreted through the kidneys (cf. calcium phosphate casts).

The patient's condition had become more and more unsatisfactory as a result of anorexia and vomiting. Accordingly it was decided that activity must be started. Therefore, the patient was allowed to bear weight on the fractured leg and activity was forced along as rapidly as possible. In one month's time the serum calcium fell from 14.4 to 11.3 mg. per 100 cc.; the serum phosphorus fell from 4.4 to 3.6 mg. per 100 cc.; the serum phosphatase rose from 5.8 to 8.3 Bo-

Tibial biopsy. The bone biopsy taken from the tibia at the end of the first operation showed the findings which one would expect in atrophy of disuse. Thus there were no osteoclasts seen at all which is strong evidence that the pathological process was not one of increased bone destruction. One cannot be sure from the section that the osteoblasts were actually decreased in number as one might expect. It is, of course, possible that the osteoblasts were there in normal numbers but were less active in laying down extracellular substance.

Calcium excretion in the urine. The calcium excretion in the urine was studied on the metabolic ward from Nov. 9 to Nov. 17, 1940. It will be seen from figure 4 that this period coincides with a time when he was already well on his way to recovery. The ab-

normalities, therefore, were probably not so extensive as they had been previously. During three 3-day metabolic periods while on a neutral low calcium intake (591 mg. of calcium per 3-day period) he excreted in the urine 1390 mg., 1234 mg., and 1030 mg. of calcium respectively. This is a tremendous increase above the normal. Thus, Farquharson, Salter, Tibbets and Aub (7) using only a slightly lower calcium intake, found for normal adults an average 3-day calcium excretion in the urine of 190 mg.

DISCUSSION

Hypercalcinemia is not a feature of the osteoporosis associated with senility, the post-menopausal state, or Cushing's syndrome. It is probably only met with in osteoporosis in young, active individuals in whom a large part of the skeleton is suddenly immobilized either by a plaster cast or as a result of infantile paralysis or some similar condition. Whether the atrophy of bone seen with nerve injuries is entirely due to disuse rather than to the absence of some trophic influence is beyond the scope of this discussion.

The question of whether a change in the balance of calcium occurs following immobilization of a large part of the human skeleton has been previously investigated on the metabolic ward of this hospital. A boy of 16 (*case 1*) with scoliosis was studied by Farquharson, Salter, Tibbets and Aub (7) before and after being placed in a plaster shell. The immobilization produced no significant change in the balance of calcium. A boy of 14 with a congenital dislocation of the hip was observed by Bauer and Ropes (8) before and after an open reduction. His pelvis and legs were immobilized in a plaster spica following operation. There was an increase in the negative balance of calcium from 0.03 gm. in 3 days during the control period before operation to 0.98 gm. in 3 days, a month after operation. No alteration in the blood levels of calcium and phosphorus occurred. The reason that these two boys did not show the change in metabolism and blood levels of calcium shown by the patient reported in this paper lies probably in the difference in degree of activity before immobilization of the skeleton. Our patient was an active, healthy boy. The two boys previously studied were inactive because of their lesions even before complete immobilization. Their bone cortices were thin and their trabeculae were not abundant. Immobilization resulted, therefore, in the release of relatively little calcium.

It is the authors' opinion that the renal insufficiency was the result and not the cause of the hypercalcinemia; however, once the renal insufficiency had asserted itself, it probably increased the tendency to hypercalcinemia.

That hypercalcinemia can be associated with osteoporosis is certainly not generally recognized. However, Dr. William J. Orr of Buffalo, New York, in a personal communication to one of the authors stated that he found hypercalcinemia in ten of eleven patients with poliomyelitis and extensive paralysis. His interest in the subject started with a most interesting case which has many points in common with the case here reported and which, with his permission, is here abstracted.

The patient, a boy of 9 years, developed extensive anterior poliomyelitis during August, 1939. Four months after onset he became delirious and was admitted to the Buffalo Children's Hospital where he had repeated convulsions. His blood pressure was found to be consistently elevated (140 to 170 mm. Hg, systolic). His urine showed white cells, occasional red cells, many granular casts (? calcium phosphate casts), a positive test for albumin, and a specific gravity between 1.010 and 1.020. The serum calcium level was 18.9 and 18.7 mg. per 100 cc.; the serum phosphorus was 6.9 and 4.8 mg. per 100 cc.; the serum protein was 7.4 gm.% The spinal fluid calcium level was 9.8 mg. per 100 cc. During the subsequent months the serum calcium level gradually returned to normal.

The decision to mobilize our patient was also supported by hearing from Dr. John E. Howard of Baltimore (9) that he and Dr. Lawson Wilkins had had similar experiences in young individuals with infantile paralysis.

That young individuals may develop hypercalcinemia with osteoporosis of disuse has more aspects of interest than the academic one of not confusing the condition with hyperparathyroidism. It is believed that the hypercalcinemia in such cases might even lead to a fatal issue either as a result of kidney damage or as a result of symptoms due to the hypercalcinemia per se.

SUMMARY AND CONCLUSIONS

1. Osteoporosis is defined as that category of decreased bone mass where the primary disturbance is an underactivity of the osetoblasts in laying down organic matrix; it is, therefore, primarily a disorder of tissue metabolism and only secondarily one of calcium metabolism.

2. Stresses and strains probably constitute the normal stimulus for osteoblastic activity; atrophy of disuse, a special form of osteoporosis, is thought to be the result of a deficiency of this normal stimulus.

3. Associated with osteoporosis, one usually expects to find normal serum calcium, normal serum phosphorus, and normal serum phosphatase levels; hypercalcinuria, however, occurs in the early stages and may lead to renal complications.

4. A case is cited of an adolescent boy where the sequence of events was probably the following: *a*) solitary bone cyst; *b*) fracture through cyst; *c*) immobilization of a large part of a previously active

skeleton; *d*) osteoporosis of disuse; *e*) an excess of calcium from the bone being presented to the kidneys for excretion; *f*) an inability of the kidneys to excrete promptly all the calcium; g) resulting hypercalcinemia; and, *h*) kidney damage from excess of calcium being excreted through the kidneys.

5. The presence of hypercalcinemia in the case just cited led to the erroneous diagnosis of hyperparathyroidism.

6. From the experience with this patient and from similar findings of other investigators it is concluded that immobilization of a large part of the skeleton in adolescent individuals may result in hypercalcinemia associated with a rapidly developing osteoporosis; this hypercalcinemia may not only lead to the erroneous diagnosis of hyperparathyroidism but may also produce a fatal issue because of kidney damage or because of the symptoms from the hypercalcinemia per se.

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Linear Growth and Epiphyseal Closure

[Bone Growth]

Effect of Treatment with Chorionic Gonadotropic Substance—A Report of 23 Cases

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IN RECENT YEARS the use of chorionic gonadotropic substance in the treatment of various pathological conditions occurring in children has increased tremendously. This increase became especially marked when concentrated preparations began to be used for the treatment of cryptorchidism, gonadal hypoplasia, and genital hypoplasia. Since then many conflicting reports have been published relative not only to the efficacy of chorionic gonadotropic substance in these particular conditions, but also regarding the possible harmful effects of its use on bone growth especially in regard to early epiphyseal closure.

Animal experimentation has indicated a definite relationship between the administration of active hormonal agents and growth of skeletal tissue. That such a direct relationship does exist has been recognized over a long period of time.

The effect of estrogens on skeletal growth has been studied by many workers. Recently Pfeiffer and Gardner (1), working with pigeons, have reported that injections of estrogen to the extent of 1,000 I.U. daily for 10 weeks will produce a rise in serum calcium with hypercalcification of the marrow-containing bones which occurs at the time of ovulation in the female, and in the male after about 5 weeks. Seeman (2), in 1937, working with lipoid estrogenic extractions of hog testes, reported premature closure of epiphyses in guinea pigs. The material assayed about 40 M.U. per cc. Talbot (3), injecting estrogen into immature rats for from 7 to 9 days following birth, reported that there was a significant advance in skeletal age which applied particularly to the female. Estrogen in daily dosage of 12.5 micrograms in 0.1 cc. of sesame oil was administered. Silberberg and Silberberg (4), studying the action of estrogen on skeletal tissues in the guinea pig, noted vague changes in the epiphyseal lines at three days, becoming more definite after one week's treatment. They describe the response of the cartilage cells as "temporary cessation of growth and differentiation, followed by degenerative changes and subsequent proliferation and hypertrophy. . . . These processes may be considered as premature aging of the cartilage." Gardner and Pfeiffer (5), in 1939, showed increased ossification in the marrow cavities of the femurs, calvaria, and other bones in mice following injections of estrogen. When testosterone propionate was given at the same time, the estrogenic effect was nullified. Weekly doses of 100 to 1,000 I.U. of estrogen were given in conjunction with from 0.625 to 2.5 mg. of testosterone propionate weekly. Other reports have appeared in which the consensus of the above findings has apparently been substantiated.

However, accurate and systematic studies in the human subject regarding the effects of the various hormones on skeletal tissue growth and bone age have been relatively few.

Epiphyseal closure is commonly believed to follow sexual maturity within definite time limits. The advanced bone age and tendency toward early epiphyseal closure is a well recognized finding in the dysfunction of macrogenitosomia praecox due to pineal or adreno-cortical tumors. There is also early closure of the epiphyses in states of hypergonadism. Hence such individuals, on reaching adulthood are below the average in height. On the other hand, the retardation of bone age and late closure of the epiphyses in the eunuchoid individual are also well known. Such delay in closure of the epiphyses in the eunuchoid or markedly hypogonadal patient is usually accompanied by an extreme increase in height.

It was probably because of this type of relationship that the suggestion was offered in the past that extreme care should be exercised in order to prevent

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epiphyseal closure in patients being treated with gonadotropic substances. Engelbach and Schaefer (6), in 1934, suggested that there exists an antagonism between the sex and growth factors. The fear of the production of dwarfism has led many clinicians to assume an ultra-conservative attitude, and in many instances, has undoubtedly prevented the use of adequate dosage of chorionic gonadotropin.

feminine characteristics both in their structural build and in their personality traits.

The length of treatment varied from a minimum of 5 months to a maximum of 24 months.

A commercial preparation[1] of a concentrated chorionic gonadotropic substance containing 500 R.U. per cc. was used. The total amounts used varied greatly, ranging from 3000 R.U. to 90,000 R.U. Naturally treatment was discontinued as soon as the desired effects on the genitalia were obtained. In the majority of the cases, the injections were given twice a week. However, some of the children reported but once a week for treatment. The average dose was 500 R.U., although in a few instances, 750 R.U. were given at each treatment.

TABLE 1. EFFECT OF TREATMENT WITH CHORIONIC GONADOTROPIC SUBSTANCE ON LINEAR GROWTH

Case	Age	Length of Treatment	Total Dosage	Height, Inches				Normal Expected Growth	Gain or Loss Over Expected Growth	
				At beginning of treatment		At end of treatment				
				Actual	Average for age	Actual	Average for age			
	yr., mo.	mo.	R.U.					in.	in.	%
1, (E.W.)	10, 3	13	16,750	51	54½	53¾	56¾	2¼	+ ½	22.2
2, (S.M.)	9, 7	14	40,750	54½	53¾	59¾	56½	3¼	+2	61.5
3, (H.R.)	15, 1	20	25,500	61½	65¼	66	67¼	2½	+2	80.0
4, (E.H.)	10, 7	24	85,500	58½	55⅜	64	59⅜	4	+1¾	43.7
5, (W.C.)	12, 7	22	20,250	58¾	59⅜	64½	64	4⅜	+1⅛	24.3
6, (L.S.)	12, 0	10½	8,750	52¼	58	54¼	59¾	1¾	+ ¼	12.8
7, (D.B.)	12, 5	13½	20,800	60	59	65¾	61½	2½	+3¼	130.0
8, (G.D.)	14, 7	18	43,000	59¾	64¾	64¼	67¼	2½	+2	80.0
9, (R.J.)	11, 6	11½	28,500	56⅜	57	59¼	59	2	+ ½	25.0
10, (F.W.)	10, 5	7	21,000	56½	54¾	57¼	55¾	1	− ¼	−25.0
11, (W.H.)	14, 0	5	3,000	59¾	63	61	63¾	¾	+ ½	66.7
12, (W.H.)	7, 5	8	16,000	50½	48¾	53¾	50¼	1½	+1¼	116.5
13, (R.H.)	8, 8	10	12,000	54	51¼	57	52⅜	1½	+1½	100.0
14, (W.T.)	8, 11	5	19,750	56	52	56½	52¼	¾	− ¼	−33.3
15, (B.R.)	11, 0	20	90,000	59¼	56	62	59¼	3¼	−1	−27.2
16, (D.G.)	11, 3	6	23,000	59¾	56½	60¼	57½	1	− ½	−50.0
17, (B.S.)	10, 5	6½	25,000	56⅜	54¾	58	56	1¼	+ ⅜	30.0
18, (D.S.)	9, 10	7	34,000	56	53¾	57½	54¾	1	+ ½	50.0
19, (L.M.)	5, 6	7½	40,000	43¼	45	45¼	46¼	1¼	+ ¾	60.0
20, (R.S.)	6, 7	8½	32,000	45¾	47⅛	48	48½	1⅜	+ ⅞	63.6
21, (C.S.)	7, 11	21	42,000	50½	50	53⅝	53⅜	3⅝	0	0
22, (L.M.)	11, 8	10	43,000	56	57½	57¾	59	1½	+ ¼	16.7
23, (C.C.)	13, 8	5	16,500	64	62	65½	63½	1½	0	0

That this fear may be ungrounded was recently expressed by Dorff (7) in his observations on the effects of chorionic gonadotropic substance on the height and osseous development in sexually underdeveloped young boys. He found "no evidence of premature union or closure of the epiphyses or termination of growth in height," following its use.

With the above in mind, a series of boys suffering from cryptorchidism, genital hypoplasia and gonadal hypoplasia were selected and treated with chorionic gonadotropic substance. The effect of the treatment on linear growth and the status of the epiphyses were carefully noted.

The series consisted of 23 cases. The age range was from 5 years and 6 months to 15 years and 1 month. In 15 of the cases, both testes were undescended, and in 3 only one was undescended. In 3 cases, there was marked genital and gonadal hypoplasia. In the remaining 2 cases, the genitalia were normal, but the boys were physically underdeveloped and showed many

The height of every child was recorded at the beginning of treatment, at the end of treatment and in a certain number of cases, months after the treatment had been discontinued. Similarly the bone age of the majority of these children was determined by means of roentgenograms of the epiphyses at the beginning of treatment, and also at the end of treatment.

Table 1 records the chronological age of the child

[1] The chorionic gonadotropin used in this study was Antuitrin-S (Apoidin) and was supplied by Parke, Davis & Company Detroit, Mich. The potency of the solution of chorionic gonadotropin used was adjusted to contain 500 R.U. per cc., or an equivalent of 600 I.U.

in years and months, the length of treatment in months, the total dosage used, the actual height and the average medium height for that age at the beginning and end of treatment, the normal expected increment in growth for the period of treatment, and the gain or loss above this both in inches and percentage for that period. An analysis of this table shows that every child gained in height. For the series as a whole, there was an average increase over the expected gain in height for the period of treatment of 36.9%. This suggests a possible stimulation of skeletal growth by chorionic gonadotropic substance. Of the 23 cases, 17, or approximately 74%, showed a gain over the expected normal increment in the rate of growth ranging from a minimum of 12.8 to a maximum of 130%. In 2 cases, the growth just equalled the average rate, while in 4 cases, or 17%, there was evidently a slowing up in the rate of growth ranging from 21 to 50%.

A number of questions immediately arise. What effect did the height of the individual at the time of beginning of treatment have on these results? Similarly, what effect did the total dosage used and the length of time of treatment have on these results?

In the group of 17 boys who showed a gain in height above the expected normal increment for the particular ages, 8 were under the average in height, 6 were above the average in height, and the remaining 3 were approximately average in height at the time treatment was started. However, the increase in growth above the normal expectancy was greater in those who were above the average in height at the time the treatment was started (78.4%) than in those who were under the average in height when treatment was commenced (50.0%). On the other hand, an analysis of the 4 cases that gained less than the expected rate of growth, shows that they were markedly above the average in height for their ages at the time treatment was started. Hence, on the basis of the results of the group as a whole, the factor of being over or under height at the beginning of treatment can be eliminated as a factor having any material bearing on the results.

Eight of the 17 patients who showed an increase over the expected increment in growth were treated for comparatively short periods of time, namely 10 months or less. The extra gain in height in these 8 cases ranged from 16.7 to 116.5% or an average of 63% compared with 58% for the group of 17 as a whole. In other words, although treated for a shorter period, they showed a greater percentage increase in height above the expected norm than those treated for longer periods of time. However, the obvious inference from this statement appears to be negated by the results in the 3 cases who failed to grow at the expected rate, and who were also treated for very short periods of time, namely 5, 6, and 7 months respectively. This is beautifully illustrated in *cases 16* and *17*. Although both of these boys were treated for approximately the same period of time, namely 6 months, and received approximately the same dosage, the results were entirely different. In the one case (*case 16, D.G.*), there was a loss of 50% in the normal expected increment of growth while in the other case (*case 17, B.S.*) there was an increase of 30% above the normal expected rate of growth. Thus, the length of time of treatment also apparently played no significant rôle in the results that were obtained.

The average dosage in the 23 cases was approximately 31,000 R.U. In the group of 4 cases, who showed a loss in relation to the expected normal increment, the dosage varied from 19,750 R.U. to 90,000 R.U., or an average dosage of approximately 33,400 R.U. In the 2 cases where the growth equalled the expected normal rate, the average dosage was 29,250 R.U., and in the remaining 17 cases where the rate of growth was greater than the expected normal, the average dosage used was slightly above 29,100 R.U. In other words, the difference in dosage in relation to the results obtained in growth was apparently of no significance in this series of cases.

Another point of interest is the possible correlation of the age of the child at the beginning of treatment, the dosage used, the length of time of treatment, and the final result in terms of linear growth. With this in mind, the cases were divided according to age into two groups. In the first group were placed all the children whose ages ranged from 5 years and 6 months to 12 years, a total of 16 cases. In the second group, were placed all those who were 12 years or more at the time treatment was commenced, a total of 7. Twelve years was selected as the dividing line because of the usual onset of puberty at that age. From table 2 it will be seen that the average dose employed in the first or younger group was 35,620 R.U.; the average length of treatment was 11.2 months, and the average gain over the expected increment in growth was 22.7%. In the group of older children the average dosage was 19,700 R.U.; the average length of treatment was 13.4 months, and the average gain in height above the normal expectancy was 56.2%.

Thus the 2 groups were treated for approximately the same length of time. The dosage in the older group was but half (55%) of that used in the younger group. However, the average gain in height above the normal expectancy was 31% greater in the older than in the younger group of children.

In other words, the gonadotropic substance accelerated the normal rate of growth in both groups. This, however, was more pronounced in the group of older children despite the smaller dosage used, leading to the belief that the effect of the gonadotropic sub-

stance probably increased the normal stimulus that puberty itself is known to exert on bone growth.

TABLE 2. CORRELATION OF AGE AT BEGINNING OF TREATMENT AND THE EFFECT ON LINEAR GROWTH

Case No.	Age yr., mo.	Dose R.U.	Length of Treatment mo.	Gain (or Loss) Over Expected Growth per cent
		UNDER 12 YEARS		
1	10, 3	16,750	13	+ 22.2
2	9, 7	40,750	14	+ 61.5
4	10, 7	85,500	24	+ 43.0
9	11, 6	28,500	11½	+ 25.0
10	10, 5	21,000	7	− 25.0
12	7, 5	16,000	8	+116.5
13	8, 8	12,000	10	+ 10.0
14	8, 11	19,750	5	− 33.3
15	11, 0	90,000	20	− 27.0
16	11, 3	23,000	6	− 50.0
17	10, 5	25,000	6½	+ 30.0
18	9, 10	34,000	7	+ 50.0
19	5, 6	40,000	7½	+ 60.0
20	6, 7	32,000	8½	+ 63.6
21	7, 11	42,000	21	0
22	11, 8	43,000	10	+ 16.7
Av.	9, 4	35,620	11.2	+ 22.7
		OVER 12 YEARS		
3	15, 1	25,500	20	+ 80.0
5	12, 7	20,250	22	+ 24.3
6	12, 0	8,750	10½	+ 12.8
7	12, 5	20,800	13½	+130.0
8	14, 7	43,000	18	+ 80.0
14	14, 0	3,000	5	+ 66.7
23	13, 8	16,500	5	0
Av.	13, 5	19,700	13.4	+ 56.2

Table 3 presents the results of the effect of the treatment on the bone age in 15 cases of this series. In these cases, the bone ages were determined by means of roentgenograms of the centers of ossification both at the beginning and at the end of treatment. From this table it is apparent that there was an increase in bone age over the normal expectancy in only 5 cases. In one case there was less than the normal expectancy in increase in bone age. The remaining 9 cases followed the normal expectancy in regard to increase in bone age. In other words, 60% of the cases maintained the normal increment of growth in respect to the bone age. This fact is very interesting, when considered in connection with the results shown in table 1, where a definite increase in height over the normal increment in rate of growth was demonstrated in 74% of cases. These two findings, when taken together, appear to prove that the chorionic gonadotropic substance stimulated growth without hastening the closure of the epiphyses, thus resembling the type of growth seen in eunuchoid states. In the latter, the overheight is due to increase in the length of the bones while the bone age as determined by roentgenogram of the epiphyses is equal to or even less than the chronological age. Similarly, in this series of cases, there was an increase in linear height without a corresponding advance in the bone age in the majority of the cases. In fact, in only one case of the entire series (*case 2*) was the bone age greater than the chronological age at the end of treatment.

Age at the time of commencement of treatment with respect to the effects on the epiphyses as shown by roentgenograms apparently was no factor. The average age of the 9 children who showed no change

TABLE 3. EFFECT OF TREATMENT WITH CHORIONIC GONADOTROPIC SUBSTANCE ON BONE AGE

Case No.	Age	Length of Treatment	Total Dosage	Age				Change in Bone Age	Gain (or Loss) Over Expected Increment
				At beginning of treatment		At end of treatment			
				Chronological	Bone age	Chronological	Bone age		
	yr., mo.	mo.	R.U.	yr., mo.	yr.	yr., mo.	yr.	yr.	yr.
1	10, 3	13¼	16,750	10.3	7	11.4½	9	2	+1
2	9, 7	14	40,750	9.7	8–9	10.9	12	3–4	+2
3	15, 1	20	25,500	15.1	12	16.9	15	3	+1
4	10, 7	24	85,500	10.7	9	12.7	12	3	+1
5	12, 7	22	20,250	12.7	9	14.5	12	3	+1
6	12, 0	10½	8,750	12.0	10	12.10½	10	0	−1
7	12, 5	13½	20,800	12.5	11	13.6½	12–13	1–2	0
8	14, 7	18	43,000	14.7	12	16.1	14	2	0
9	11, 6	11½	28,500	11.6	8	12.5½	9	1	0
10	10, 5	7	21,000	10.5	8	11.2	9	1	0
11	14, 0	5	3,000	11.9[1]	9[1]	14.5	11–12	0	0
12	7, 5	8	16,000	7.5	7	8.1	7–8	0	0
13	8, 8	10	12,000	8.8	8	9.6	9	1	0
14	8, 11	5	19,750	8.11	9	9.4	9	0	0
23	13, 8	5	16,500	13.8	14	14.1	14	0	0

[1] Two years before treatment began.

in the expected bone age was 11.3 years, while the average age of the 5 boys in whom there was an increase in bone age over the normal expectancy, was 11.6 years. However, as noted above, the age at which treatment was commenced was of importance in determining the maximum effects of the gonadotropic substance on linear growth. Thus, in discussing the effects of chorionic gonadotropic substance on bone growth and structure, we must differentiate between its effects on linear growth and its effects on bone age as determined by epiphyseal development.

The rôle that the dosage played in producing these results is questionable. The 5 cases showing an increase in the bone age over the normal increment were in the group receiving the larger dosages. From this, therefore, it might be argued that the larger the dosage, the greater the effect on the bone age, and therefore on the possibility of hastening the closure of the epiphyses. However, closer analysis shows that the one case in the group of 5 which received the largest dose, namely 85,500 R.U., showed less of a gain over the normal increment than the one receiving

TABLE 4. LINEAR GROWTH AFTER DISCONTINUING TREATMENT

Case No.	Height at End of Treatment	Length of Time Treatment Discontinued	Present Height	Expected Increment of Growth	Gain (or Loss) Over Expected Increment of Growth	
					Inches	Per cent
	in.	*mo.*	*in.*	*in.*		
3	66	9	$67\frac{1}{4}$	$\frac{3}{4}$	$+\frac{1}{2}$	66.7
5	$64\frac{1}{2}$	6	66	$1\frac{1}{4}$	$+\frac{1}{4}$	20.0
6	$54\frac{3}{4}$	23	$60\frac{1}{4}$	$5\frac{1}{4}$	$+\frac{3}{4}$	14.3
8	$64\frac{1}{4}$	14	$66\frac{1}{2}$	$1\frac{1}{4}$	$+1$	80.0
11	61	20	66	$3\frac{1}{4}$	$+1\frac{3}{4}$	54.8
14	$56\frac{1}{2}$	18	$58\frac{1}{2}$	$2\frac{3}{4}$	$-\frac{3}{4}$	−27.2
15	62	14	$65\frac{1}{2}$	3	$+\frac{1}{8}$	4.2
18	$57\frac{1}{2}$	5	$58\frac{1}{2}$	$\frac{3}{4}$	$+\frac{1}{4}$	33.3
19	$45\frac{1}{4}$	7	$47\frac{3}{8}$	$1\frac{1}{4}$	$+1\frac{1}{4}$	100.0
20	48	$13\frac{1}{2}$	52	$2\frac{1}{4}$	$+1\frac{3}{4}$	77.8
21	$53\frac{5}{8}$	7	$55\frac{3}{4}$	$1\frac{3}{8}$	$+\frac{3}{4}$	54.5
22	$57\frac{3}{4}$	5	$58\frac{1}{8}$	1	$-\frac{5}{8}$	−62.5

half this amount. Similarly, the group in which there was no increase over the normal increment received equally large doses in the same percentage of cases. Hence, judging from this series of cases, the amount of gonadotropic substance used bore no relationship to the effects noted on the bone age. This is in agreement with the deduction made from table 1, namely, that the dosage used bore no relationship to the effect in the increase in the linear growth.

The length of time of treatment, however, may have been a factor in producing the results on the centers of ossification noted above. The 5 cases in which there was an increase in the bone age above the expected norm were treated for a much longer period of time than the remaining 10 cases, the average length of time being 18.6 months for the former and 9.2 months for the latter. Here again the difference in the effects of another constant factor, namely the length of treatment, on linear growth and bone age is striking. Whereas this constant factor has apparently no effect on the former, it does seem to have an effect on the latter. This should make one cautious regarding the prolonged use of chorionic gonadotropic substance regardless of the dosage employed or the age of the patient.

In 12 cases of the series it was possible to note the results, as far as linear growth was concerned, after

TABLE 5. COMPARISON OF EFFECT ON LINEAR GROWTH DURING AND AFTER TREATMENT

Case No.	Gain (or Loss) Over Expected Increment of Growth (in per cent)	
	During treatment	After treatment
3	80.0	66.7
5	24.3	20.0
6	12.8	14.3
8	56.6	80.0
11	66.7	54.8
14	−33.3	−27.2
15	57.0	4.2
18	50.0	33.3
19	60.0	100.0
20	63.6	77.8
21	0	54.5
22	16.7	−62.5

treatment had been discontinued. The periods of observation ranged from 5 to 23 months. The results are presented in table 4. From this table it will be seen that with the exception of 2 cases, all of the boys gained more than the expected increment for the particular period of time following cessation of treatment. This gain above the normal rate varied from 14.3% in *case 6* to 100% in *case 19*. These cases showed the same comparable growth above the expected norm after treatment was discontinued as during the course of treatment. This is shown in table 5.

In but 4 of the twelve cases were there large variations, and in these, the variations were of such a nature as to demonstrate that the gonadotropic substance was not the responsible factor in the results obtained since in 2 of the 4 cases, the gain above the expected increment was greater during the course of treatment, and in the other 2 cases it was greater after treatment was discontinued.

Of the two cases that did not gain in height more than the expected increment following cessation of treatment, one (*case 14*) showed a loss of 27.2% under the expected rate of growth and the second (*case 22*) showed a loss of 62.5%. It is interesting to note that *case 14*, while under treatment, also showed

a similar loss (33.3%). This would lead to the belief that in this case, the gonadotropic factor had no influence as a growth stimulant. It should be noted that in this case also the boy was 4 inches above the average in height at the beginning of treatment. Eighteen months after treatment was discontinued, he was still almost 3 inches above the average in height.

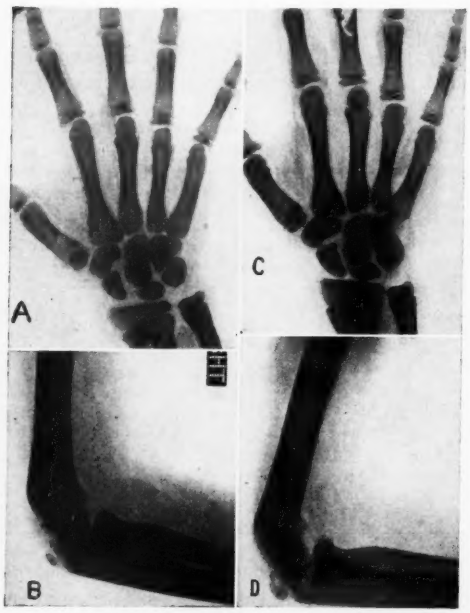

Fig. 1. HAND AND ELBOW OF W. T. (*case 14*), AGE 8 YEARS, 11 MONTHS. *Plates A* and *B* show a bone age of 9 years at the beginning of treatment. *Plates C* and *D* show the same bone age after receiving 19,750 R.U. of chorionic gonadotropic substance for 5 months.

Case 22 was apparently the only exception in this series of cases. Although there was a 16.7% gain above the expected rate during treatment, in the 5 months following discontinuance of treatment, there was a loss of 62.5% from the expected rate of growth.

In general, judging from the above results it would appear justifiable to assume that either the chorionic gonadotropic substance was not the only factor causing the acceleration in growth or the accelerating effects of the hormonal substance continued after treatment was stopped. Again both of these assumptions may be correct in that the continued acceleration in growth present in this series of cases may have been due to the indirect stimulation by the gonadotropic substance of other endocrine glands.

One statement can be made with a fair degree of certainty, namely, that the use of gonadotropic substance in this series of cases did not interfere with the growth of these children when treatment was discontinued.

Although not germane to the problem under investigation, the results of the treatment with the gonadotropic substance on the genitalia of these children are worth mentioning.

Of the 15 boys in whom both testes were undescended, descent of both testicles occurred in 13 cases, descent of one testicle in one case, and no results in one case.

Of the 3 boys in whom only one testicle was undescended, descent occurred in all.

The three cases that were treated for gonadal and genital hypoplasia showed growth to normal size.

It is interesting to note that both boys, who received treatment because of their physical underdevelopment and feminine characteristics, showed a marked improvement in both respects. They gained appreciably above the expected increment in linear growth and the secondary sex characteristics became more pronounced. They also became more masculine in their behavior.

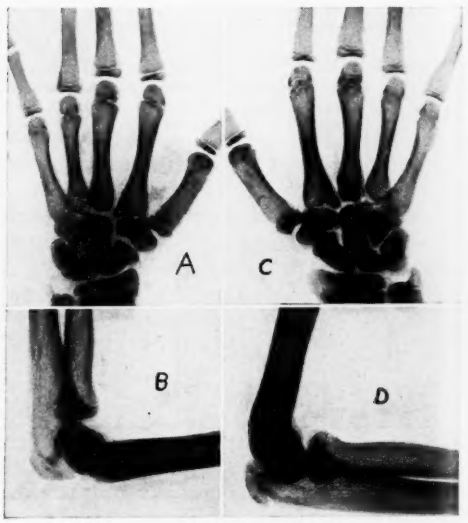

Fig. 2. HAND AND ELBOW OF G. D. (*case 8*), AGE 14 YEARS, 7 MONTHS. *Plates A* and *B* show a bone age of 11 to 12 years at the beginning of treatment. *Plates C* and *D* show a bone age of almost 14 years after receiving 43,000 R.U. of chorionic gonadotropic substance over a period of 18 months.

The following 4 cases are cited as examples:

W.T. (*case 14*) age 8 years, 11 months, was treated for unilateral intra-abdominal cryptorchidism and genital hypoplasia. Treatment lasted 5 months during which time he received 19,750 R.U. of chorionic gonadotropic substance. In addition the boy re-

ceived desiccated thyroid extract grain ½ *t.i.d.* At the beginning of treatment, he was 56 inches tall (4 inches above the average for his age) while at the end of treatment, he was 56½ inches tall (3¾ inches above the average for his age). The expected gain for this period of time was ¾ inches. Thus the boy gained 33.3% less than the expected amount. The bone age at the end of treatment was the same as at the beginning of treatment, namely, 9 years (fig. 1). The one undescended testicle was delivered into the scrotum and the penis increased in size. Eighteen months after treatment was discontinued, the boy was 58½ inches tall. This represented a gain of 2 inches in height but a loss of ¾ inches (27.2%) of the normal expected growth.

C.D. (*case 8*) age 14 years, 7 months, was treated for genital hypoplasia, over a period of 18 months during which time he received 43,000 R.U. of gonado-

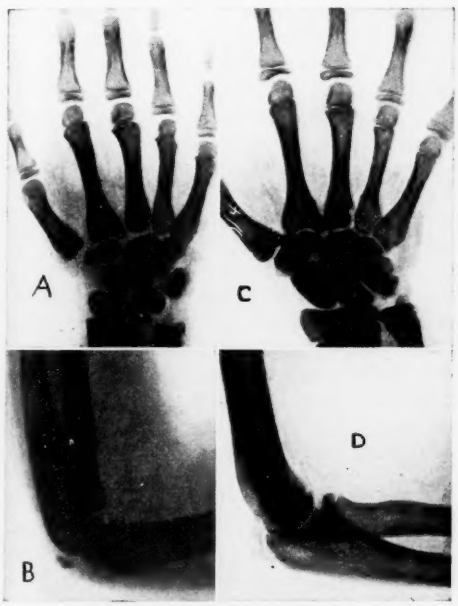

Fig. 3. HAND AND ELBOW OF E. H. (*case 4*), AGE 10 YEARS, 7 MONTHS. *Plates A* and *B* show a bone age of 9 years at the beginning of treatment. *Plates C* and *D* show a bone age of 12 years after having received 85,500 R.U. of chorionic gonadotropic substance over a period of 24 months.

tropic substance. He also received desiccated thyroid extract grain ½ three times a day. At the beginning of treatment, he was 59¾ inches tall (5 inches under the average for his age) and at the end of treatment, he was 64¼ inches tall (3 inches under the average for his age). This represented a gain of 1⅝ inches, or 56.6% above the normal increment in growth for that period. In terms of bone age there was a gain of 2 years, from 12 to 14, as shown in figure 2. The genitalia became normal in size and the secondary sex characteristics became manifest. Fourteen months following discontinuance of treatment, the boy was 66½ inches tall which represented a gain of 1 inch or 80% above the normal expectancy in rate of growth for that period.

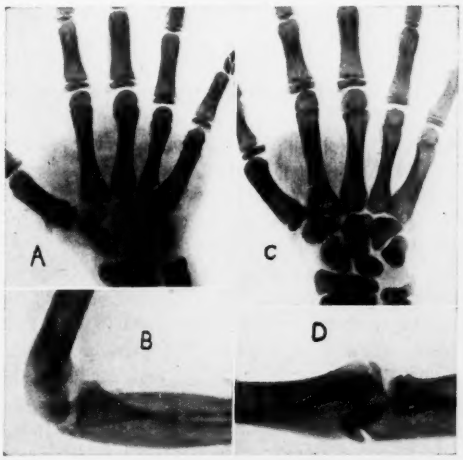

Fig. 4. HAND AND ELBOW OF S. M. (*case 2*), AGE 9 YEARS, 7 MONTHS. *Plates A* and *B* show a bone age of 9 years at the beginning of treatment. *Plates C* and *D* show a bone age of 12 years after having received 40,750 R.U. of chorionic gonadotropic substance over a period of 14 months.

E.H. (*case 4*), age 10 years, 7 months, was treated for bilateral intra-abdominal cryptorchidism. The penis was hardly visible. He received 85,500 R.U. of chorionic gonadotropic substance over a period of 24 months. In addition he received ½ grain of desiccated thyroid extract three times a day. His height at the beginning of treatment was 58½ inches (2⅞ inches above the average for his age). At the end of treatment he was 64 inches tall (4⅝ inches above the average for his age.) In other words, the boy gained 1¾ inches or 43.7% above the normal expectancy in growth for that period. The bone age at the beginning of treatment was approximately 9 years and the end of treatment was about 12 years, representing a gain of 1 year or 50% above the expected rate of growth in bone age (fig. 3) On completion of treatment, the penis was normal in size and the testes were in the scrotum.

S.M. (*case 2*), age 9 years, 7 months, was treated for bilateral cryptorchidism and infantile penis. He received 40,750 R.U. of gonadotropic substance during a period of 14 months. At the beginning of treatment he was 54½ inches tall (¾ inch above the average),

while at the end of treatment he was 59¾ inches tall (3½ inches above the average). In other words, the boy gained 2 inches, or 61.5% above the normal expected rate in growth. Similarly he gained approximately 3 years in bone age above the normal expectancy (fig. 4). This was the only case in the entire series in which the bone age was greater than the chronological age at the end of treatment. Both testes descended into the scrotum and the penis became larger.

SUMMARY

Twenty-three boys suffering from cryptorchidism, genital hypoplasia, and gonadal hypoplasia were treated with chorionic gonadotropic substance. The age range was from 5 years, 6 months to 15 years, 1 month; the length of treatment varied from 5 to 24 months; and the dosage used varied from a minimum of 3,000 to a maximum of 90,000 R.U.

For the series as a whole there was an average increase over the expected gain in linear height for the period of treatment of 36.9%. Seventeen (74%) of the 23 cases showed a gain over the expected normal increment of growth; 2 (9%) just equalled the normal gain, while 4 (17%) gained less than the normal expectancy. Neither overheight nor underheight at the beginning of treatment, nor the length of time of treatment, nor the dosage employed had any material bearing on these results.

When the cases were divided according to age into 2 groups using 12 years as the arbitrary point of division because of its relationship to the onset of puberty, it was noted that the gonadotropic substance accelerated the normal rate of growth in both groups. However, this effect was more pronounced in the group of children above the age of 12. This despite the fact that they received but half the dosage of gonadotropic substance that was employed in the group under the age of 12. A deduction that appears justifiable from this is that the gonadotropic substance probably increased the normal stimulus that puberty itself is known to exert on bone growth.

In 15 cases of the series, the bone ages were determined by roentgenograms both at the beginning and the end of treatment. Nine of these cases (60%) maintained a normal increment of growth in respect to bone age. One case gained less than the normal, while 5 (33.3%) showed a gain in bone age over the normal expectancy. This finding is very striking when compared with the fact that 74% of the entire series of cases showed a gain in linear height above the normal expectancy. In other words, the results here noted corresponded to those usually seen in cases of eunuchoidism where there is an increase in linear height without a corresponding increase in bone age. In only one case of the entire series was the bone age greater than the chronological age at the end of treatment. Furthermore, while the age of the child at the time treatment was commenced was a factor in determining the effect on linear growth, it had no effect on bone age as determined by epiphyseal development. The dosage used also bore no relationship to the effects noted on the bone age. However, the length of time of treatment may have played a rôle in the results on epiphyseal growth or closure. The 5 cases in which there was increase in the bone age above the expected norm were treated twice as long as the remaining cases. This should make one cautious regarding the prolonged use of chorionic gonadotropic substance regardless of the dosage employed or the age of the patient.

Twelve of the patients of this series were observed for periods of 5 to 23 months after treatment was discontinued. All but 2 of the boys gained more than the expected increment in the rate of growth. The gain ranged from 14.3 to 100%. Judging from this, it would appear justifiable to assume that either the chorionic gonadotropic substance was not the only factor causing acceleration in growth or that its accelerating effects on bone growth continued after treatment was stopped. In any event, it can be stated definitely that the use of gonadotropic substance in this series of cases did not interfere with later growth when treatment was discontinued.

The results of the treatment with chorionic gonadotropic substance on the genitalia of these children may be stated briefly as follows: of the 15 boys in whom both testes were undescended, descent of both testicles occurred in 13 cases, descent of one testicle in 1 case, and no change occurred in one case. Of the 3 boys in whom only one testicle was undescended, descent occurred in all of the three. The 3 cases that were treated for gonadal and genital hypoplasia showed growth to normal size.

CONCLUSIONS

In evaluating the effects of chorionic gonadotropic substance on bone growth, it is essential to differentiate between the effects of this substance on linear growth and on epiphyseal growth or closure. Heretofore, most authors in their experiments with this substance have failed to recognize the existence as well as the importance of this differentiation. To the majority of workers in this field, gain in height was synonymous with increase in bone age as determined by the status of the centers of ossification. As a result, an increase in height following the use of chorionic gonadotropic substance was interpreted to mean that there was a corresponding increase in bone age. Hence the fear of premature closure of the epiphyses seemed to be well founded.

The present report emphasizes the need for this

differentiation, as the findings indicate varying results in respect to these two factors.

As far as linear growth is concerned, treatment with chorionic gonadotropic substance showed an average gain in height above the normal expectancy of 36.9% in 74% of the cases. However, in 60% of the cases, the growth in bone age only equalled the normal expectancy. Again the age at which treatment was commenced played a definite rôle in the results noted on linear growth but had no effect as far as the bone age was concerned. On the other hand, while the duration of the period of treatment did not influence the results in regard to linear growth, it bore a direct relationship to the gain in bone age.

Finally, we feel justified in concluding that the use of chorionic gonadotropic substance in this type of case is not dangerous as it neither produces premature closure of the epiphyses nor retards linear growth after treatment has been discontinued. The one note of warning that should be sounded is that this substance should not be used over too long a period of time.

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Von Recklinghausen's Disease with Diabetes Mellitus

[Neurofibromatosis and Diabetes]

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This case is reported because of the unusual finding of diabetes mellitus with Von Recklinghausen's disease. A survey of the literature has failed to find mention of these two conditions associated in one individual. Fairly complete reviews of Von Recklinghausen's disease are available (1–7).

The term, Von Recklinhausen's disease, is preferred to neurofibromatosis by many authors. This seems justifiable because the condition appears to be a complex syndrome which may manifest itself in one or more different forms. It is unfortunate that the term, Von Recklinghausen's disease, is also applied to osteitis fibrosa cystica. Makism (5) lists the following forms in which the disease may make itself evident: (*a*) formes frustes (incomplete) showing café au lait spots, (*b*) multiple neurofibromatosis (cutaneous, subcutaneous, peripheral nerve, cranial nerve, spinal nerve or sympathetic nerve tumors), (*c*) neurofibromatosis with osseous changes, (*d*) elephantiasis neuromatosa, (*e*) mental changes and (*f*) endocrine changes. One or more of these may be present and often in the absence of all but the café au lait spots one is hard pressed to make a diagnosis. The family history may be of value since Preisor and Davenport showed that about 43% of their cases were on a heredo-familial basis. They believed the disease to be inherited as a dominant characteristic.

CASE REPORT

A white boy, 12 years old, was referred by his local physician because of a slowly growing tumor on the lower back, which was first observed about two years before admission. The growth of the tumor had been continuous. It had never appeared to be inflammatory in nature and there had never been symptoms referable to it. The past history revealed severe malnutrition with rickets during infancy and the usual childhood diseases. The patient was said to be "nervous, cries easily," and did poorly in school. Systemic review was non-contributory with no symptoms or signs suggesting diabetes mellitus.

The family history is not complete because the father was vague about some of his distant relatives. However, the father was certain that the patient's paternal great-grandmother had "brown spots and small lumps all over her body." The paternal grandparents had no apparent stigmata. The father has typical neurofibromatosis with café au lait spots. Two paternal uncles and two paternal aunts have similar stigmata. Another paternal uncle may have had similar manifestations. The patient has had "brown spots on his skin" since birth. The patient's brother has similar "spots" but the youngest child, a girl, is apparently free of any manifestations. The mother's side of the family is free of signs suggesting the disease.

Physical examination showed a fairly well-developed, thin, white boy who appeared to be about twelve years old. He was cooperative and affable. No secondary sex characteristics were present. Ht. was 5′5½″ (166 cm.); head, 21½″ (55 cm.); chest 25″ (63.5 cm.); abdomen 28″ (71 cm.). Wt. was 59 lb. (26.8 kg.). The skin over the trunk and extremities was covered with many irregularly shaped café au lait spots of various sizes. In the region of the first lumbar vertebra, there was a soft, easily movable mass, which measured 5 × 3 × 2 cm., lying under the skin. This was not tender, red, nor warm. There were numerous smaller tumors on the back and upper extremities. The thorax showed a marked sternal depression with evidence of healed rickets. Neurological examination was negative. It is interesting to note here that the father had a similar sternal depression.

Laboratory data were as follows: red blood cells, 5.02 m; hemo. 14.5 gm.; white blood cells 9,550; polymorphonuclear leucocytes, 90; lymphocytes, 8; monocytes, 1; eosinophils, 1; mantoux test, 1:1,000, negative; 1:100, slightly positive; Schick test, positive. Lumbar puncture test result was negative. Stools contained neither ova nor parasites. Hinton test result was negative. Routine urine analysis showed a specific gravity of 1.040, acid reaction, 2 plus sugar, no albumin, acetone, or abnormal microscopic findings. Blood chemistry showed: fasting sugar 97 mg.%; urea nitrogen 16.9 mg.%; CO_2 content 58.8 vol.%; phosphorus 4.3 mg.%; calcium 9.5 mg.%; serum proteins 8.4 gm.; albumin 4.8 gm.; globulin 3.6 gm.; cholesterol 196 mg.%; B.M.R. +22%; repeated in duplicate 2 days later B.M.R. was +5, +3, using Child Research Council standards. Bromsulphalein test showed no dye in the serum at the end of ½ hour. Glucose and galactose tolerance tests are shown below. X-rays of skull, sella, long bones, wrists, and spine were non-contributory except for spina bifida occulta of the first sacral vertebra. Revised Stanford-Binet Test (Scale M) showed a mental

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age of 10 years 3 months as compared with a chronological age of 12 years, 1 month, giving an intelligence quotient of 85 with a normal scattering of successes and failures between 9 and 13 years.

Glucose tolerance test, using 50 gm. of glucose

	Blood sugar mg.%	Urine sugar gm.%
Fasting	80	0
30 minutes	380	0.25
1 hour	222	3.0
2 hours	125	trace
3½ hours	91	0

Substance in urine identified as glucose by fermentation, Seliwanoff, and osazone tests.

Exton-Rose 1-hour 2-dose glucose tolerance test

(25 gm. given after fasting blood sugar specimen was taken, and 25 gm. at end of ½ hour)

	Blood sugar mg.%	Urine sugar gm.%
Fasting	95	0
½ hour	147	0.25
1 hour	233	0.25
2 hours	139	0.25
3 hours	91	0

Galactose tolerance test, 30 gm. galactose

	Reducing substance in urine	
	10/12/40 gm.	10/15/40 gm.
Fasting	0	0
1 hour	1.5	0
2 hours	1.6	0
3 hours	0	0
4 hours	0	0
Total	3.1	0

Course in hospital. The presence of a 2+ sugar in the admission urine analysis lead to a search for an explanation. Casual urine specimens showed from 2+sugar to no sugar, and had a specific gravity varying from 1.040 to 1.002. Since the glucose tolerance test gave a most unexpected result, it was repeated, using the Exton-Rose 1-hour, 2-dose test. This also gave a diabetic type of curve. Accordingly the patient was placed on a weighed diet which contained 2,000 calories of which 50% was carbohydrate, 15% protein, and 35% fat, divided equally among the 3 meals. Urine specimens were collected from 9 P.M. to 6 A.M., 6 A.M. to 11 A.M., 11 A.M. to 4 P.M., and 4 P.M. to 9 P.M. Sugar was never found in any of these specimens except on one occasion. This occurred on the day breakfast was delayed by a basal metabolism test. The boy finally received his breakfast at 10:30 A.M., and lunch at noon. The 11 A.M. to 4 P.M. specimen had 0.5% sugar. The tumor of the back was removed surgically and found to be characteristic of Von Recklinghausen's disease on microscopic examination.

DISCUSSION

Our patient showed four of the more common characteristics of Von Recklinghausen's syndrome, namely: café au lait spots, tumors, mental and endocrine changes. The finding of the spina bifida occulta is not the characteristic osseous change as described by Brooks and Lehman (8).

The etiology of Von Recklinghausen's disease is still unknown. It has been considered to be an heredofamilial defect of the germ plasm. Gordon (3) is of the opinion that the endocrine disturbances are part of the syndrome but not the etiological factors. All of the endocrine glands have been reported to be involved with the single exception of the pancreas. The endocrine glands, according to Gordon, may be involved by actual ingrowth of the tumor or by functional changes with or without anatomical alterations. In our patient, the only endocrine gland which can be shown to be involved is the pancreas.

The rapid disappearance of the glycosuria when the patient was placed on a weighed diet and the frankly diabetic nature of the glucose tolerance curves, point strongly to a diagnosis of early diabetes. It is recognized that such factors as previously low carbohydrate diets, infections, pituitary disease and hyperthyroidism occasionally give rise to a diabetic type of curve. Our patient, however, was on a general ward diet for several days prior to the tests and showed no demonstrable evidence of infection or glandular disease primarily involving any organ other than the pancreas. Contrary to earlier belief, it is acknowledged that the early manifestations of juvenile diabetes may not be striking and may pass unnoticed until the condition is well established. In mild cases which are not accelerated through infectious disease, the progression of the typical symptoms may be slow. In fact, in early diabetes there may be considerable inconstancy of the sugar-handling function, hyperglycemia and glycosuria being followed after a few days by hypoglycemia. What connection the pancreatic disturbance may have with Von Recklinghausen's disease is purely a matter for conjecture. The few cases of this condition in which glucose tolerance tests were reported showed normal curves.

SUMMARY

A case of Von Recklinghausen's disease with diabetes mellitus in a 12-year-old boy is reported. This apparently is the first time the two conditions have been reported in the same individual.

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Effect of Various Gonadotropins upon Spermatogenesis and Urinary Androgens in the Human[1]

[Spermatogenesis]

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The purpose in this clinical investigation was to study and to compare the effects of the administration of various gonadotropins upon human spermatogenesis and urinary androgen excretion. The materials used in the survey consisted of chorionic gonadotropin,[2] to be designated as APL, pregnant mare serum derivative[3] (PMS), and anterior pituitary extract (AP), which contains primarily the gonadotropic element, as stated by the manufacturer. The subjects comprised 14 male patients, for whom repeated examinations of semen and repeated assays of urinary androgens, either one or both, were done during the period of investigation. The presenting symptoms and final diagnoses varied in these cases, and will be discussed subsequently.

Since gonadotropic response in the human so often differs from that found in animal experimentation, and because the effects in animals are generally well known, we shall confine our review of the literature to a partial survey of reported clinical findings.

Heckel (1) found an increase in spermatogenesis in 3 of 12 cases during the administration of Antuitrin. Pregnancy occurred in two instances. Four of 8 patients improved while receiving Antuitrin-S, and again pregnancy occurred in 2 cases. Kreutzmann (2) observed the development of aspermia in a patient with 34% abnormal forms after receiving 0.5 cc. of chorionic gonadotropin 3 times a week for a month. In another case, 26% abnormal spermatozoa were demonstrated on a semen examination before treatment, and an increase to 74% abnormal forms occurred after the use of 2 cc. of pregnant mare serum derivative 3 times weekly for a month. He states that the only consistently good results with endocrine therapy occur in those cases in which thyroid function is deficient. Meaker and Vose (3) condemn the routine use of gonadotropic substances in cases of infertility, and emphasize the beneficial effects of thyroid substance in hypometabolic cases. Rubinstein (4) studied the spermatogenic responses to Follutein or Antuitrin-S in 6 healthy adult males. No effect upon morphology, motility, or viability was observed. The total counts increased after 4 to 7 weeks, and remained high for 2 to 5 weeks after cessation of treatment in 5 cases. Hotchkiss (5) reported an increase in the number, motility, and viability of spermatozoa in 3 cases after the administration of anterior pituitary extract. Pregnancy occurred in 2 of the cases. Wilhelm (6) treated 6 cases of nonobstructive azoöspermia with APL without improvement. In 17 cases, consisting of necrospermia, oligospermia, or both, an apparent increase in numbers and motility occurred in 6 instances. However, he feels that improvement in none of these cases could be definitely attributed to APL. Of 27 cases treated with a combination of APL and AP, or with the latter only, 11 showed an increase in the number and motility of the sperm, and pregnancy resulted in 5 cases. Improvement occurred in 4 of 6 cases treated with PMS. He noted a reduction in the number of sperm cells in cases treated over long periods of time, which possibly were due to antihormone or antibody formation. Thomas (7) reported the occurrence of pregnancy in 65% of 83 cases of sterility (after excluding those due to azoöspermia and obstruction of the fallopian tubes). He felt that these results could not be attributed to endocrine gland therapy, except for the use of desiccated thyroid. Mazer, Israel, and Charny (8) used Prephysin, Gamone, and Antuitrin-S in 21 patients, with an increase in the number of spermatozoa in only 2 instances. Thyroid extract, exercise, and high protein diets had proved more effective.

The 14 patients whose responses to gonadotropins are discussed here may be roughly divided into 5 groups, dependent upon the presenting complaint or general clinical picture. *Group 1.* Those with clinical evidence of pituitary disorder (*cases 2 to 5*). One of

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[1] We are indebted to Dr. F. J. McGurl, W. E. Flannery and H. R. Rossmiller for cooperation in caring for many of the details in connection with administration of gonadotropins and with the collection of specimens.

[2] We wish to express our appreciation to Ayerst, McKenna, and Harrison Ltd., Rouses Point, N. Y., for their generosity in supplying us with the A.P.L. and Pituitary Gonadotrophic Factor used in this investigation.

[3] Our appreciation is due Mr. Donald Wonder of the Cutter Laboratories, Berkeley, California, for the generous supply of Gonadin used, and to Dr. Max Gilbert of the Schering Corporation, Bloomfield, N. J., for his courtesy in supplying us with the Anteron which was used in several cases.

these complained of infertility. *Group 2.* Those who complained of infertility, but who had little or no clinical evidence of disease (*cases 6 to 11*). *Group 3.* A chief complaint of infertility, but with history of x-ray exposure (*case 12*). *Group 4.* A chief complaint of relative impotence, but with little or no evidence of disease (*cases 13 and 14*). *Group 5.* A diagnosis of alopecia totalis (*case 15*). Androgenic response alone was studied in 2 cases; spermatogenic response alone was studied in 5 cases; both responses were followed in 7 cases. In 12 instances observations with two or three different gonadotropins were made in the same case.

The AP extract used in this investigation was standardized to a concentration of 300 R.U. (Collip) per cc. as supplied by the manufacturer, and is so expressed in all tables and graphs. The quantities of APL and PMS are expressed in international units (I.U.) in all tables and graphs. Where such a standard may not have been adopted until recently, an effort has been made to express previous unitage in I.U. equivalents. All preparations were administered by intramuscular injection, the quantity at any one time varying from 1 to 5 cc. Injections were given daily or on alternate days.

Semen specimens were collected in clean glass containers, usually after intercourse and occasionally by masturbation. An effort was made to assure a period of 4 to 7 days sexual continence prior to collection of each specimen. The reason for this requirement is illustrated by the following case report.

Case 1 (fig. 1). A 23-year-old male patient was seen because of a history of chronic fatigue. There were no other significant complaints. The physical examination findings were normal, except for the fact that the weight was somewhat below the average normal. Urinalysis findings were normal. There was no anemia. Blood sugar levels and blood serology were normal. The B.M.R. was −8%.

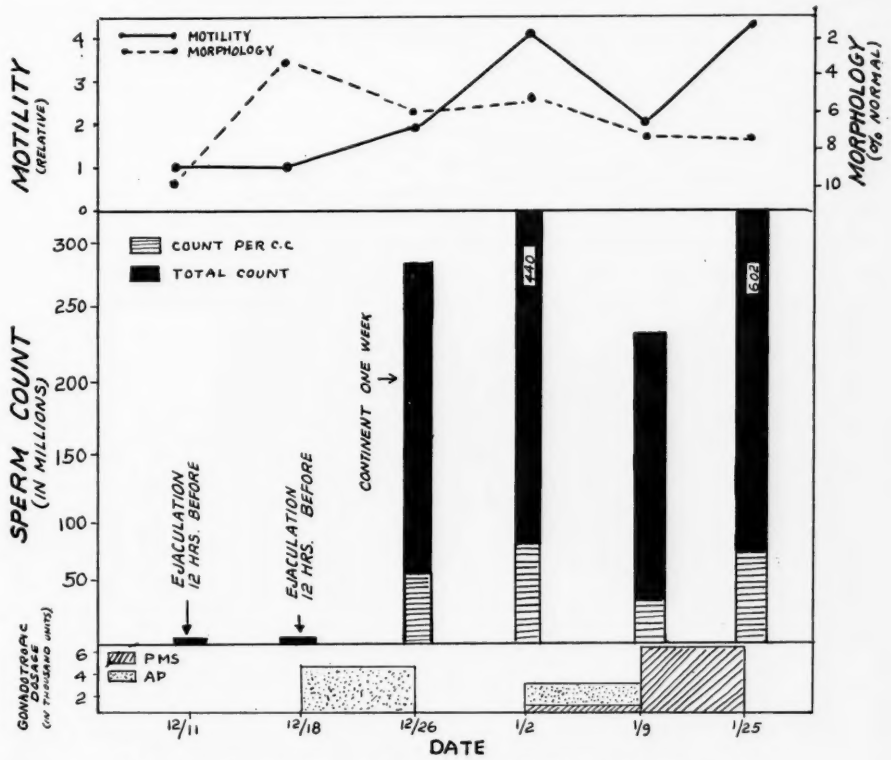

Fig. 1. SPERMATOGENIC RESPONSE TO GONADOTROPINS PMS AND AP EXTRACT, *case 1*.

In connection with our studies on spermatogenic response to gonadotropins, a semen examination was made, and an apparent marked oligozoöspermia was found. The examination was repeated with similar results, so treatment with AP extract was begun. The next specimen was normal except for a slight decrease in motility, possibly attributable to the time interval between collection and examination. Careful questioning at this time revealed the fact that ejaculation had occurred 12 hours or less before collection of each of the first two specimens, whereas sexual continence had been practiced for a week before collection of the normal specimen. Subsequent studies con-

firmed the normality of this patient's spermatogenic mechanism.

The technic of semen examination carried out in our laboratory is that described by McCullagh and Guillet (9). Some of the reports on sperm motility and morphology are approximations, since accurate determinations were impossible because of the low total sperm counts. The volume of each semen speci-

TABLE 1. ANDROGENIC RESPONSE TO GONADOTROPINS (*Case 2*)

Date	Gonadotropin	Dosage	Androgen assay
		U	I.U.
9/23/39	APL	32,500 since 4/28/39	2
9/30/39	APL	1,500	2
10/29/39	PMS	1,200	6
11/ 5/39	PMS	800	3
11/20/39	PMS	1,500	8
11/29/39	PMS	3,200	8
12/10/39	PMS	2,000	11
12/28/39	PMS	1,500	4
1/21/40	PMS	2,400	0

men has not been reported in most instances because no correlation was found between the total volume and other factors, and because of the obvious factor of error encountered in collection of a specimen after intercourse. The volume is indicated, however, on most of the graphs and tables by a report of the total sperm cell count as well as the count per cc. Viability, likewise, is not reported in this series.

The assays for urinary androgens were performed on 24-hour specimens. No fixed interval between the time of the preceding gonadotropin injection and collection of the urine specimen was followed. In those cases where both androgenic and spermatogenic responses were studied, urine for assay was not always collected on the same day as the nearest chronological semen examination. However, a comparison of response to the same gonadotropin usually was possible.

All assays were made by biological methods. The technic (9) is a modification of that described by Gallagher and Koch (10). The normal range varies from approximately 18 to 85 I.U.

The tables and graphs used in the case reports are largely self-explanatory. It should be mentioned that no effort has been made to present the time intervals in graphic form, but the dates are stated. In those graphs where urinary androgen studies are shown in addition to semen examinations, the date of collection of the urine specimen is indicated by a figure close to the androgen level. Where only a small percentage of spermatozoa have been found to be alive at the beginning of a semen examination, the percentage figure is shown close to the motility indicator on the graph. On all graphs and tables the dosages of APL and PMS are expressed in I.U., and the dosages of AP extract are expressed in R.U.

The following is a more detailed presentation of each case. The blood count, urinalysis, blood sugar, and blood serology were normal in each instance and need not be repeated in the individual case reports.

CASE REPORTS

Case 2 (table 1). This male patient, the first of a series of 14 on whom gonadotropin response was studied, was 18 years of age. The height was 52.8 inches, and the weight was 72 pounds. There was no goiter. He did not have the appearance of hypothyroidism. The genitalia were puerile, and there was no evidence of puberty. Skeletal maturity in April 1939 was calculated to be between 8 and 9 years. Roentgen examination of the sella turcica in 1933 showed it to be normal.

Desiccated thyroid had been used in varying dosages to tolerance for most of the time since 1933. The B.M.R. estimated repeatedly over a period of years, had been found to be as low as -28%.

The presence of exceedingly low levels of urinary androgens (table 1) indicates a failure of androgen production by both the testes and the adrenals. Under such circumstances, it might seem reasonable to conclude that the diagnosis of pituitary deficiency is corroborated. Theoretically, if these considerations be true, this is the type of case which should respond very actively to gonadotropic substances. Instead, there was an insignificant response in urinary androgen excretion following the use of both APL and PMS in the doses shown.

Case 3 (table 2). This was a 22-year-old male patient with a chief complaint of sexual underdevelopment. A

TABLE 2. ANDROGENIC RESPONSE TO GONADOTROPINS (*Case 3*)

Date	Gonadotropin	Dosage	Androgen assay
		U	I.U.
9/ 9/39	None	—	26
10/23/39	None	—	24
10/30/39	PMS	2,000	19
11/ 6/39	PMS	2,400	36
11/14/39	PMS	400	69
11/20/39	PMS	1,600	30
12/11/39	PMS	9,200	36
12/18/39	PMS	3,200	32
1/ 8/40	AP	7,800	14
1/22/40	APL	1,800	51
2/12/40	APL	3,900	32
3/11/40	APL	13,200	15
4/17/40	APL	16,000 to 4/4/40	38

history of left testicular injury at 14 years of age was elicited. No genital growth had been observed during the 8 years preceding admission. Erections occurred rather frequently, but were not maintained, and there had been no ejaculations. Hair growth on all parts of the body had been scant. Body growth had occurred at a normal rate.

The height was 67.3 inches and the weight was 118 pounds. Bodily proportions were eunuchoid. A downy hair growth was present on the face. Scant hair growth

was present on the extremities. Axillary hair was estimated to be one-fourth that of normal individuals and pubic hair one-half that of normal males. The penis was 5 cm. in length. The right testis measured 1.0 cm. by 0.6 cm. The left testicle was in the inguinal canal, and was estimated to be somewhat smaller than the right. The prostate was scarcely palpable. The remainder of the physical examination disclosed no significant abnormality.

The blood cholesterol was 161 mg. per 100 cc., one hour and 15 minutes postprandial. The B.M.R. prior to admission was −29%. Dessicated thyroid was not used. Roentgenographic study of the skull showed the sella turcica to be normal. Skeletal development, roentgenographically, was normal for 17 years of age.

and the weight was 206 pounds. Hair distribution was normal. The voice was high-pitched. The testes and prostate were normal. The penis was smaller than the average normal. The remainder of the examination was non-contributory. The B.M.R. was −6%.

Prior to initiation of any treatment, the sperm cell count was normal, although motility was slight and there was a high percentage of abnormal forms (table 3). The use of testosterone propionate resulted in a striking decrease in this count, as would be expected from previous reports (11). The administration of 5,600 I.U. of PMS within a period of one month did not alter the inhibitory effect of the

TABLE 3. SPERMATOGENIC RESPONSE TO GONADOTROPINS (*Case 4*)

Date	Treatment	Dosage	Count per c.c.	Total count	Motility (relative)	Morphology (% normal)
3/23/38	None	—	192,000,000	960,000,000	<1+	97
4/20/38	None	—	120,000,000	720,000,000	<1+	93
5/11/38	Testosterone propionate	225 mg.	111,000,000	588,000,000	<1+	95
7/20/38	Testosterone propionate	750 mg.	5,600,000	21,840,000	None	90
8/10/38	Testosterone propionate	225 mg.	900,000	2,970,000	None	—
9/ 7/38	PMS	5,600 I.U.	Too few to count		None	—
10/13/38	None	—	Too few to count		None	—
11/23/38	None	—	360,000,000	2,340,000,000	3+	50
1/ 4/39	PMS	8,400 I.U.	150,000,000	750,000,000	2+	40
3/13/39	Testosterone propionate	750 mg.	Average of 4 per low power field		None	—

During the administration of PMS in the dosages indicated in table 2 there was a rise in the level of urinary androgens to as high as 69 I.U. from previously low levels. This rise was not maintained while under treatment with the same substance, although subsequent levels were well within normal range. After the injection of AP extract in total dosage of 7,800 R.U. within one month, the assay for urinary androgens showed only 14 I.U. A transient rise to as high as 51 I.U. occurred during the administration of APL, followed by a decrease to pretreatment levels, with a subsequent rise to a normal level during continued administration of this material.

The responses to PMS and APL as demonstrated here might not be considered significant in a normal adult male, but are probably of importance in an individual with clinical hypogonadism.

Case 4 (table 3). A 41-year-old male patient complained primarily of deficient genital development and the presence of homosexual interests. He had been obese throughout his life, and the distribution of adipose tissue had involved particularly the mammary and pubic areas, the abdomen, the buttocks and the trochanteric areas. Erections and emissions had occurred normally, but had decreased in frequency following injection therapy for hernia. The belief that his genitalia were small had resulted in a marked psychoneurosis.

The height was 68.3 inches, the span was 68.3 inches,

testosterone propionate. Subsequent withdrawal of treatment with PMS for 2½ months resulted in a return to levels of sperm counts which were higher than those previously encountered. There were also favorable changes in the motility and morphology of the sperm. It is unlikely that the PMS, which had not been used for 2½ months was a factor in this improvement. The use of PMS during the next 1½ months had no significant effect. Testosterone propionate was again given with resultant marked depression in spermatogenesis.

If one accepts the premise that androgens depress spermatogenesis by inhibiting the pituitary, then gonadotropin administration after cessation of testosterone propionate therapy would be expected to result in a rapid return to normality. Such was not the case in this instance.

Case 5 (fig. 2). This 36-year-old, healthy adult male complained primarily of sterility. Impregnation had been attempted unsuccessfully for 4 years. A deficiency in spermatogenesis had been known to be present for almost as long, and treatment had been given in the form of one grain of desiccated thyroid daily for a year, and small and intermittent doses of pregnancy urine extract and pregnant mare serum derivative. There was no history of orchitis, testicular injury, or venereal disease.

The height was 77 inches and the weight 201.5 pounds. The features were suggestive of acromegaly, although the

condition did not appear to be progressive. The genitalia were normal, except for the fact that the right testis was soft and small, measuring 3.5 by 1.5 by 1.5 cm. There was a small adenoma of the right lobe of the thyroid. The remainder of the physical survey revealed negative findings.

A roentgenogram of the skull showed the sella turcica to be normal. The B.M.R. was −2%.

PMS was given from Oct. 2 to Nov. 27 in the doses indicated in figure 2. No increase in spermatogenesis was noted. Studies of morphology were impossible because of the low counts. Motility studies may be considered of questionable significance for the same reason. They appear to show that there was little or no motility most of the time, and that the slight increase was not maintained.

The levels of urinary androgens during the same interval appear to show a distinct change (fig. 2). The pretreatment level was definitely below normal. During the administration of PMS, there was a consistent rise, and normal levels were maintained until some time after this treatment was withdrawn. In short, it appears that in this instance there was a definite increase in androgen production but no increase in gametogenesis while PMS is used.

After a rest period of 2 weeks, treatment with AP extract was begun on Dec. 11, 1939, and continued to July 6, 1940. After 1 month, a striking increase in spermatogenesis was noted and was maintained for a period of 3 months. At no time, however, did the semen become normal. Toward the end of the third month there was a diminution in the sperm cell count to the original low level in spite of an increase in the dosage of gonadotropin. During this same interval, there appears to have been some increase in motility which did not reach normal, and which diminished before the diminution in the sperm cell count was obvious. While the count was still comparatively high, motility virtually fell to zero. Such extremely low motility however was found only once in conjunction with a relatively high sperm count.

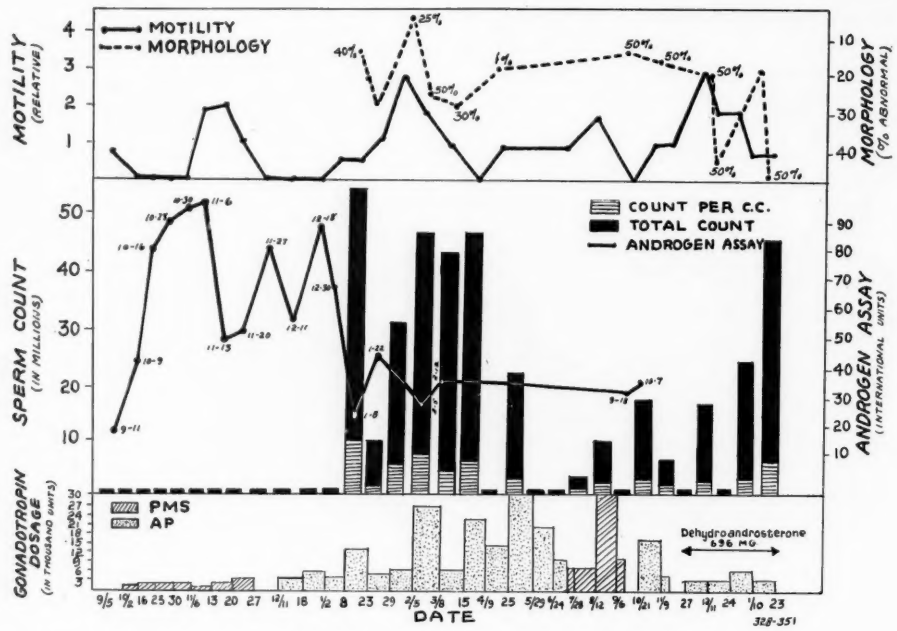

Fig. 2. Spermatogenic and androgenic response to gonadotropins PMS and AP extract, *case 5*.

During this period of administration of AP extract the androgens were at levels approximating the low range of normal or below, and they showed no response such as that which occurred while PMS was used.

Thus, it appears that in this individual, AP extract elicited a definite gametogenic response with little or no stimulation of androgen production, whereas PMS stimulated androgen production without evidence of increased gametogenesis.

Subsequent trials with PMS, as shown in figure 2 elicited little response in spermatozoa production, whereas the further use of AP extract and dehydroandrosterone together resulted again in increased spermatogenesis. Continued use of AP extract was

followed by another fall in the sperm cell count to nearly zero. At this point, a series of immature rats were injected with AP extract alone and with AP extract plus the patient's serum. The size of the uteri and ovaries of the animals receiving AP extract alone averaged approximately 4 times that of animals receiving both materials. See figure 2A. This would indicate that the patient's serum now contained a substance antagonistic to this anterior pituitary preparation.

Dehydroandrosterone was used in the hope that an increased spermatogenic effect might be obtained. It has been shown (12) that this androgen has a relatively greater spermatogenic effect and lesser androgenic effect in the hypophysectomized rat than have other androgens.

Case 6 (fig. 3). A 33-year-old male complained of sterility. His physical development and general health had been normal. He had been married for 12 years and was normally potent. No history of testicular trauma, orchitis, or venereal disease was obtained.

General physical examination revealed entirely normal findings. Careful survey in the Department of Urology revealed no organic disorder of the genital tract. The B.M.R. was -4%.

The total period of observation while the patient was under treatment was 13 months. Oligozoöspermia was present. After the administration of 11,400 I.U. of PMS in a period of 8 weeks, a noticeable numerical increase in sperm cells occurred (fig. 3). With some variation, this increase was maintained

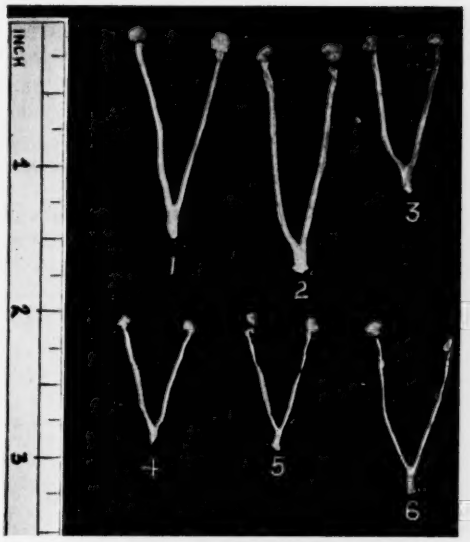

Fig. 2A. *1, 2, 3,* UTERINE AND OVARIAN RESPONSE IN 21-DAY-OLD RATS FOLLOWING ADMINISTRATION OF 10 R.U. OF AP. (Collip) daily for 3 days in each instance. *4, 5, 6,* UTERINE AND OVARIAN RESPONSE IN 21-DAY-OLD LITTER MATES OF THE ABOVE RATS FOLLOWING ADMINISTRATION OF THE SAME SUBSTANCE, PLUS 0.5 CC. OF THE PATIENT'S SERUM.

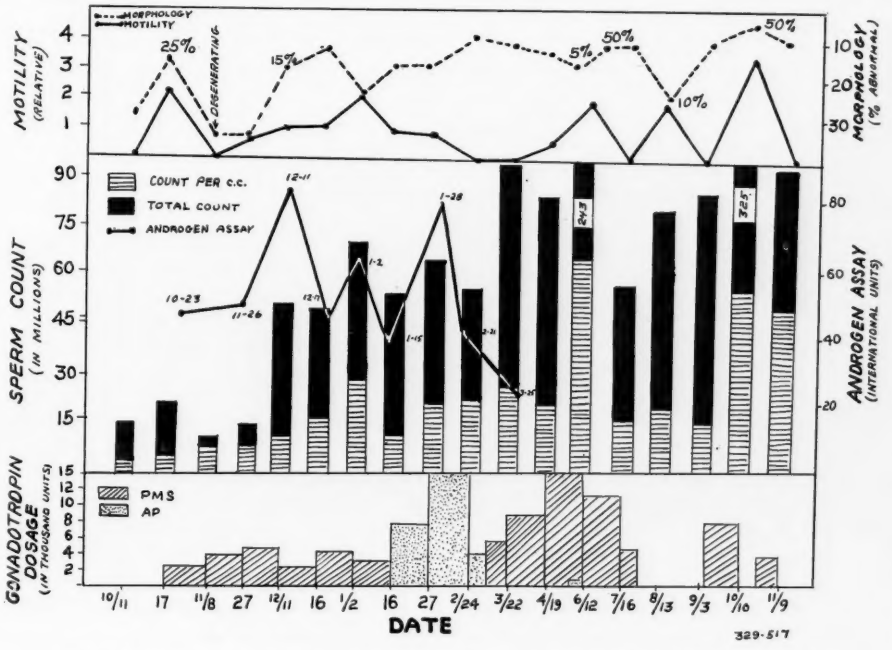

Fig. 3. SPERMATOGENIC AND ANDROGENIC RESPONSE TO GONADOTROPINS PMS AND AP EXTRACT, *case 6.*

and improved during the continued use of PMS and during a month's therapy with AP extract. The further administration of PMS, even in comparatively small dosages, resulted in numerically normal sperm studies. Motility remained below normal throughout the survey. An apparent improvement in morphology occurrred, although a majority of the spermatozoa were dead in numerous specimens.

An apparent transient increase in the levels of urinary androgens occurred during the administration of both PMS and AP extract. In an individual with

Physical development had occurred normally, and general health had been good. A first marriage had resulted in the birth of one child approximately 8 years previously. His second marriage, of 5 years' duration at the time of his examination, had been sterile. Libido and sexual potence were normal. No history of orchitis, testicular trauma, or venereal infection was elicited. Treatment prior to admission had included repeated prostatic massage, and administration of wheat germ oil, APL, orchic substance, and thyroid in small dosages.

The height was 71 inches and the weight was 146 pounds. Aside from the weight deficiency, the general

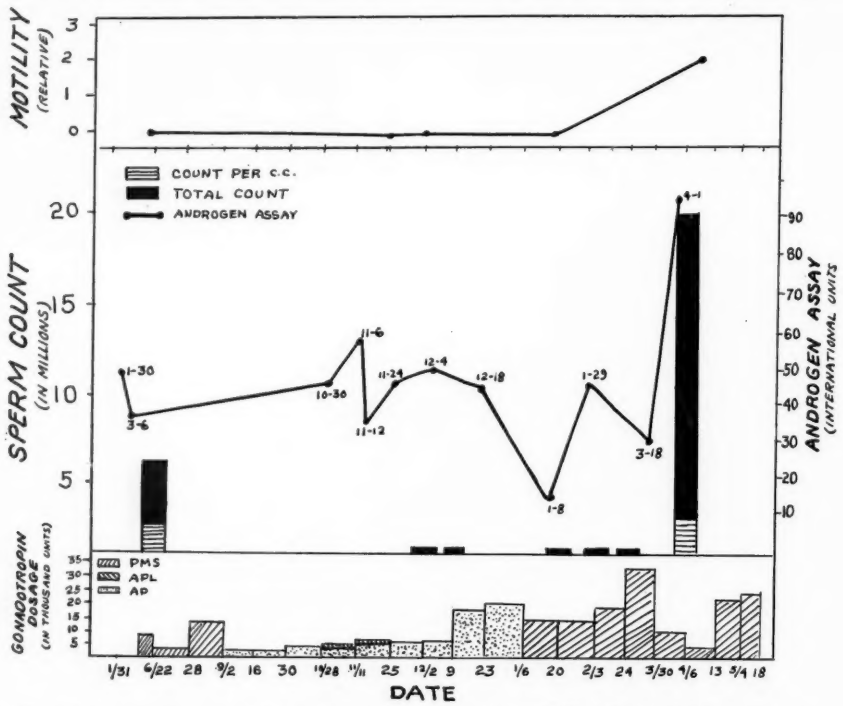

Fig. 4. Spermatogenic and androgenic response to gonadotropins PMS, APL and AP extract, *case* 7.

no clinical evidence of hypogonadism and with normal urinary androgen levels prior to treatment these apparent increases of androgens may be considered of very doubtful significance.

The wife was studied carefully in both the Endocrine and Gynecological Divisions, and no contributing factor to the infertility was found. Artificial inseminations were carried out on several occasions during midmenstrual cycles when the patient's spermatozoa approximated numerical normality, but pregnancy did not occur.

Case 7 (fig. 4). A 30-year-old male patient complained of sterility and stated that he had known of an absence of spermatozoa for two years prior to his initial visit. A testicular puncture had been done, and an "absence of sperm" reported.

physical survey was negative. The penis was of average size, and a small scar was visible on the posterior surface. Both testes were slightly smaller and of softer consistency than normal. The prostate was normal.

The B.M.R. was −8%. A roentgenogram of the skull showed a normal sella turcica.

The initial semen examination revealed a volume of 2.5 cc. and a total absence of germinal elements. Bilateral testicular punctures were made, and no active motile spermatozoa were demonstrated in the fresh wet smear. The pretreatment level of urinary androgens was 50 I.U.

Following the administration of 7,200 I.U. of PMS in 5 weeks, 2,000,000 nonmotile spermatozoa per cc. were found on semen examination (fig. 4). Further use of PMS for approximately 3 months had no effect and the azoöspermia recurred and persisted. The ad-

ministration of AP extract alone, and of AP extract in conjunction with APL in the doses indicated, likewise did not influence spermatogenesis, although a few nonmotile spermatozoa were found in two specimens. The average semen volume for determinations from June 22, 1939 to Jan. 6, 1940 was 3.0 cc. Resumption of PMS therapy resulted in the occurrence of a few spermatozoa in several specimens, and in one isolated count of 2,250,000 sperm cells per cc., with a total count of 19,800,000. Subsequent determinations during treatment showed a return to azoöspermia. Motility was zero in all determinations except that showing the highest total count, where the motility was 2+.

The levels of urinary androgens were normal before treatment and no consistent change occurred in response to gonadotropin administration. A fall to a hypogonadal range occurred following AP extract and the resumption of PMS administration. The rather striking rise on the occasion of the elevated sperm cell count during the use of PMS is probably significant and representative of definite interstitial cell stimulation.

Case 8 (fig. 5). On admission this 31-year-old male patient stated that he had been informed of a deficiency in sperm production, and wished to have the problem investigated further. He had been married for 6 years without the occurrence of pregnancy, but precautionary measures had been used during most of that time. No suggestion of androgen deficiency was obtained in the history, and libido and sexual potency were normal. He had had an apparently unilateral mumps orchitis at 15 years of age. There was no history of venereal infection.

The penis was normal. The right testis was normal in size and consistency. The left testis was small and soft. No prostatic abnormality was found. The remainder of the physical examination revealed completely negative findings.

The B.M.R. was −16%. The level of urinary androgens on admission was 44 I.U.

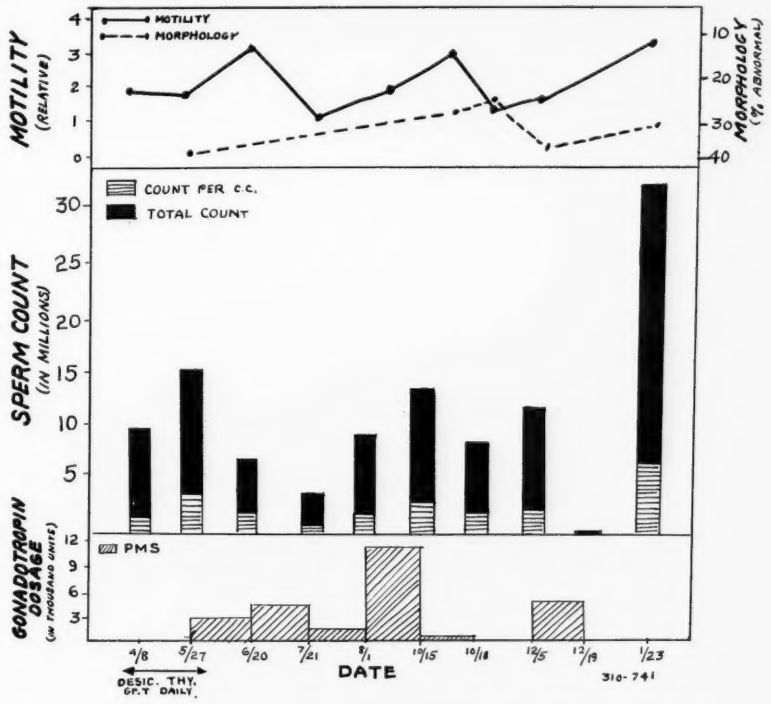

Fig. 5. Spermatogenic response to gonadotropin PMS, *case 8*.

Spermatogenic response alone was studied in this case, and PMS was the only gonadotropin used (fig. 5). A complete failure of consistent response was obtained. The highest level of 6,200,000 spermatozoa per cc. and total count of 37,200,000 was reached a month after cessation of treatment. These figures are not sufficiently high to be considered significant. Motility was below normal in all specimens. Studies of morphology were not accurate, but the percentage of abnormal forms was high in each instance.

Case 9 (table 4). A 39-year-old male patient had been

TABLE 4. SPERMATOGENIC RESPONSE TO GONADOTROPINS (*Case 9*)

Date	Treatment	Dosage	Sperm count	Semen volume	Motility
		U		cc.	
10/22/40	None	—	<1 per h.p.f.	5.8	None
11/ 5/40	None	—	<1 per h.p.f.	5.0	None
12/ 7/40	AP	4,050	None	5.5	—
12/21/40	AP	10,500	None	4.2	—
1/ 4/41	AP	10,500	<1 per h.p.f.	5.3	None
1/18/41	PMS	5,600	None	5.3	—

married for 4 years and had unsuccessfully attempted impregnation for 3 years before admission. Physical development and general health had been normal. During the past 10 years, he had had 6 or 7 attacks of 'venereal ulcer,' characterized by the occurrence of small vesicles on the shaft of the penis. Four years before admission, he had had a painful swelling in the left inguinal region, lasting for about 2 weeks. No history could be obtained of orchitis, or definite venereal infection. A bilateral herniorrhophy had been done 15 years previously. The wife had been examined elsewhere and had been reported as normal.

The penis was normal in size. A small vesicle was noted on the left lateral surface of the shaft. The left testicle was small and soft in consistency; the right testicle was normal. The prostate was normal. The remainder of the physical examination gave negative findings.

The B.M.R. was −17%. The level of urinary androgens prior to treatment was 58 I.U. A roentgenogram of the skull showed a normal sella turcica.

This case illustrates a total failure to respond to the administration of AP extract (table 4). PMS was used for only two weeks, during which time the azoöspermia persisted. One grain of desiccated thyroid daily was given during the period of treatment.

Case 10 (fig. 6). A 34-year-old male patient stated that his general health was good, but that pregnancy had been attempted unsuccessfully for 2 years. A semen examination had been made 18 months before admission, and an absence of motile spermatozoa had been reported. A course of Gynantrin and Antuitrin-S therapy had been given, after which he had been told that sperm were present and motile. When pregnancy still failed to occur, the wife was examined, and subsequently a uterine polyp was removed. Libido and sexual potency were normal. No history was obtained of testicular infection, of trauma, or of venereal disease.

General physical examination gave normal findings. The penis was of normal size, but an hypospadias with a small opening was present. The scrotal contents and prostate were normal.

The B.M.R. was +1%.

Treatment with AP extract was undertaken. A numerical increase of sperm to normal levels occurred,

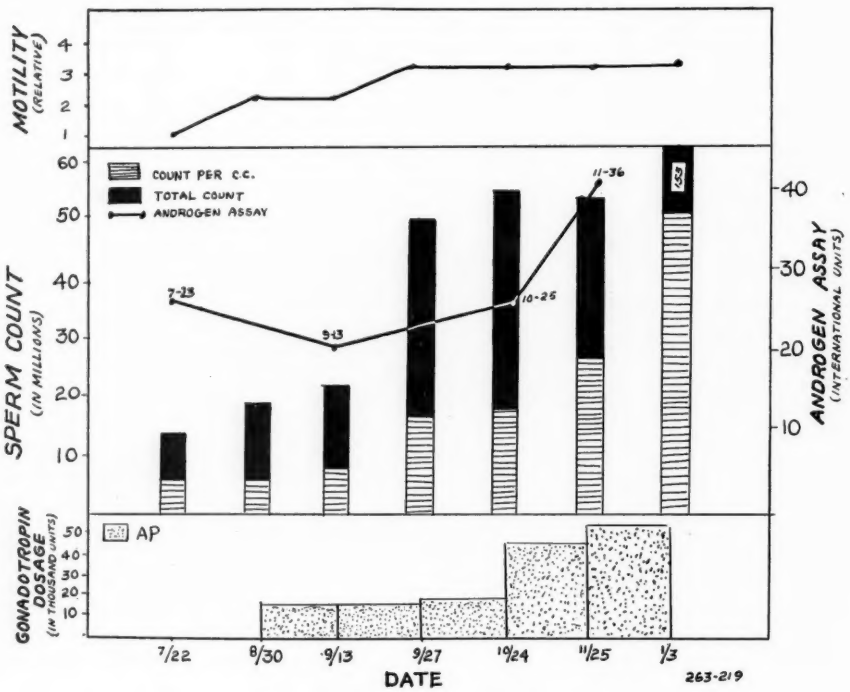

Fig. 6. SPERMATOGENIC AND ANDROGENIC RESPONSE TO GONADOTROPIN AP EXTRACT, *case 10*.

TABLE 5. SPERMATOGENIC RESPONSE TO GONADOTROPINS (*Case 11*)

Date	Gonadotropin	Dosage	Sperm per c.c.	Total count	Motility (relative)	Morphology (% normal)
		U				
10/27/39	None	—	average 4 per h.p.f.		None	—
12/16/39	PMS	4,800	4,000,000	16,000,000	1+	—
1/13/40	PMS	4,400	6,000,000	60,000,000	1+	30
2/17/40	PMS	4,800	7,000,000	59,500,000	2+	15
3/30/40	None	—	4,800,000	44,160,000	3+ (50% alive)	30

and motility likewise improved (fig. 6). Morphology was not studied. A few assays for androgenic substances were made. The level prior to treatment was normal and there was no significant change as the result of the treatment.[4]

Case 11 (table 5). This 35-year-old male patient had been married for 5 years, and pregnancy had been attempted unsuccessfully for 2½ years. Physical development had been normal, and libido and sexual potence had continued unchanged within normal limits. No history was obtained of testicular infection or trauma, or of venereal disease. General health had been good except for moderate, intermittent, generalized muscular aching. The wife had been examined elsewhere and had been reported normal. The only therapeutic agent used before admission was wheat germ oil.

The penis was normal in appearance. The testes were of average size, but of rather soft consistency. The prostate was normal in size, and was not infected. Except for a foot deformity with shortening of one lower extremity, the remainder of the physical examination was negative.

The B.M.R. was −1%. The level of urinary androgens prior to treatment was 60 I.U.

PMS was the only gonadotropin used. Relatively few observations were made, but a definite numerical increase in sperm occurred (table 5). Normal levels were not reached, but the doses used in this case were not large. Motility apparently showed some improvement. Morphology was variable.

Case 12 (table 6) A 37-year-old male patient complained of sterility. At 4 or 5 years of age, he had received roentgen therapy to the scrotum for some type of dermatitis. His voice had deepened at 16 years of age, and he had shaved first at 18 years of age. The beard, however, had remained scant, body hair had not developed in the usual amount, the skin had remained of rather fine texture, and muscular development was relatively slight. He had been married for 11 years, during 5 of which pregnancy had been unsuccessfully attempted. Libido and sexual potency were normal, although premature ejaculations occasionally occurred. There was no history of venereal infection.

TABLE 6. SPERMATOGENIC RESPONSE TO GONADOTROPINS (*Case 12*)

Date	Gonadotropin	Dosage	Sperm count	Semen volume	Motility
		U		cc.	
7/22/40	None	—	1 per h.p.f.	2.0	<1+
8/16/40	PMS	6,750	1 per h.p.f.	1.9	1+
8/21/40	PMS	2,000	None	2.1	—
10/17/40	PMS	19,000	<1 per h.p.f.	1.2	—
11/22/40	AP	12,600	None	1.0	—
1/3/41	None	—	<1 per h.p.f.	1.5	—

The physical stature was slight. The skin was of fine texture. The beard was scanty, the hair on the extremities was sparse, and the usual male escutcheon was lacking. The penis was normal in appearance. The testes were small, but of normal consistency. The prostate was about one-third to one-half normal size. The remainder of the physical survey was negative.

The B.M.R. was −20%. The level of urinary androgens was 39 I.U.

No improvement occurred with the use of PMS or AP extract (table 6). Desiccated thyroid in dosages of 1.5 grains daily was given throughout.

Azoöspermia in this case, in contradistinction to other cases, was almost certainly a result of testicular injury from roentgen therapy. Favorable response, therefore, would not be anticipated.

Case 13 (table 7 and 7A). A 30-year-old man complained of impotence. Twelve years previously he had had an unilateral mumps orchitis. Two years before admission, he had suffered a lower back strain. Relative impotence had developed shortly afterwards, and had persisted. Development had been normal. No history of venereal infection was obtained.

General physical examination revealed normal findings. The penis, left testicle, and prostate were normal. The right testicle was small and of soft consistency.

During examination in the Department of Urology, a polyp of the verumontanum was found and was subsequently removed.

The B.M.R. was determined shortly after beginning methyl testosterone therapy, and was −11% on one occasion and −9% on another. The level of urinary androgens was determined twice before beginning treatment, and was 65 I.U. in one specimen and 22 I.U. in another. Semen examination before treatment showed a moderate numerical deficiency and a decreased motility of sperm. Morphology was normal.

Following the use of testosterone propionate, depression of spermatogenesis occurred (table 7).

[4] We are indebted to Dr. H. C. Martin of Ashland, Ky., for supervising the management in this case and for reporting the findings of the semen examination.

TABLE 7. SPERMATOGENIC RESPONSE TO GONADOTROPINS (*Case 13*)

Date	Treatment	Dosage	Count per c.c.	Total count	Motility (relative)	Morphology (% normal)
		U				
6/27/39	None	—	25,000,000	187,000,000	2+ (50% alive)	20
12/13/39	Testosterone propionate	550 mg. from 10/2/39 to 11/25/39	8,000,000	40,000,000	3+ (20% alive)	5
12/20/39	APL	7,500	9,000,000	45,000,000	<1+	12
12/27/39	APL	7,500	10,000,000	29,000,000	1+	—
1/11/40	APL	15,000	8,000,000	50,400,000	2+ (50% alive)	10
1/17/40	PMS	1,600	16,800,000	84,000,000	1+ (50% alive)	9
1/24/40	PMS	5,600	16,000,000	80,000,000	2+ (50% alive)	12
2/ 2/40	PMS	7,200	22,000,000	171,600,000	1+ (50% alive)	12
2/ 7/40	PMS	4,000	46,000,000	207,000,000	2+	9
2/14/40	AP	3,600	24,000,000	115,200,000	2+ (50% alive)	6
2/21/40	AP	6,900	28,000,000	123,200,000	3+ (25% alive)	10
2/28/40	AP	8,400	24,000,000	100,800,000	2+ (75% alive)	17
3/ 6/40	AP	8,400	36,000,000	144,000,000	3+	11
3/13/40	AP	8,400	56,000,000	224,000,000	2+	7
3/20/40	AP	8,400	28,000,000	70,000,000	3+	15
4/ 2/40	Methyl testosterone	1,050 mg.	34,500,000	165,600,000	3+	25
5/15/40	Methyl testosterone	3,750 mg.	29,000,000	156,600,000	2+	14
6/26/40	Methyl testosterone	4,350 mg.	2,400,000	10,800,000	1+	10

Administration of APL for a month thereafter produced no change, and certainly did not hasten recovery. A gradual rise occurred while PMS and AP were used, but this was no greater than might be expected after withdrawal of testosterone propionate. Motility and morphology showed no significant alteration. Depression of spermatogenesis occurred again when methyl testosterone was given.

Androgen response likewise was inconclusive (table 7a). One level of 86 I.U. was obtained after the administration of only 2,500 I.U. of APL, and one level of 76 I.U. occurred after 15,200 I.U. of PMS. This patient had normal assays, including one level of 65 I.U., before treatment, so that the above elevations cannot be considered significant.

TABLE 7A. ANDROGENIC RESPONSE TO GONADOTROPINS (*Case 13*)

Date	Treatment	Dosage	Androgen assay
		U	I.U.
6/29/39	None	—	65
9/14/39	None	—	22
12/12/39	Testosterone propionate	550 mg. from 10/2/39 to 11/25/39	31
12/15/39	APL	2,500	86
12/27/39	APL	12,500	44
1/ 2/40	APL	10,000	44
1/ 9/40	APL	5,000	51
1/24/40	PMS	7,200	52
2/ 3/40	PMS	8,000	54
2/ 9/40	PMS	4,000	76
3/19/40	AP	42,900	47
4/ 3/40	Methyl testosterone	1,050 mg.	41

Case 14 (fig. 7). A 32-year-old male patient stated that erections had been unsatisfactory and ejaculations had been premature since a bilateral herniorrhophy 12 years before admission. Except for chronic constipation, he had no other complaints. Physical development had occurred normally. There was no history of venereal infection.

The left testicle was small and of soft consistency. The right testis, penis, and prostate was normal. The thyroid was slightly and diffusely enlarged. The remainder of the physical examination gave negative findings.

The B.M.R. was −9%.

The number of spermatozoa per cc. of semen was normal before initiation of treatment, although the volume was small and the motility was slight. During the use of APL, there was no significant change in the number of sperm, although motility apparently showed transient improvement (fig. 7). The level of urinary androgens was normal before treatment, but a temporary and probably significant increase occurred while APL was administered.

PMS was given for only a short period of time,

with no conclusive effect upon either semen or urinary androgen levels.

During the administration of AP extract, no conclusive alteration in spermatogenesis could be demonstrated. One urinary androgen level was increased, but not sufficiently to be considered significant.

The relative impotence was not improved to any significant degree during the use of these gonadotropins. A further decrease in potency occurred while PMS was given, but psychogenic factors were probably of primary importance.

Because of the discouraging character of this patient's problem, treatment was attempted on a frankly experimental basis. Spermatogenic and androgenic responses were followed incidentally.

Unfortunately no semen examination was made prior to initiation of testosterone propionate therapy, but some decrease in numbers of spermatozoa apparently occurred. Approximately 10 weeks after discontinuing this preparation (12/2/39), the count had increased and the depressant effect of the testosterone propionate was presumably markedly decreased

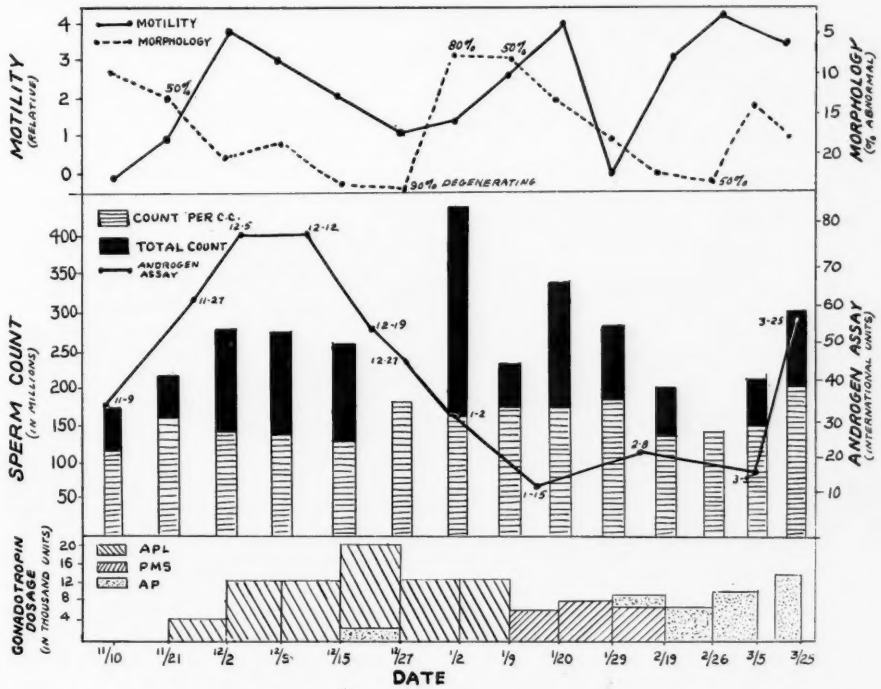

Fig. 7. Spermatogenic and androgenic response to gonadotropins APL, PMS and AP extract, *case 14*.

Case 15 (Table 8 and 8A). A 30-year-old male patient complained of total hair loss. At the age of 14 years, loss of eyebrows and eyelashes had taken place, but regrowth had occurred. Two years before admission, alopecia had recurred and had progressed to total hair loss. Libido and sexual potency were normal, and no history of genital infection or injury was obtained. Strength and energy were normal.

General physical examination showed normal findings, except for the alopecia totalis. The penis, testes, and prostate appeared to be normal.

The B.M.R. was -7%. The pretreatment level of urinary androgens was 47 I.U. A Friedman test, as a qualitative measure of pituitary function, showed no measurable amount of urinary prolan.

(table 8). The use of APL thereafter, in the doses indicated, produced no consistent increase, and certainly no change which could be attributed to the action of APL. The same negative findings occurred during the administration of PMS, although only 2 observations were made. The sharp numerical rise in sperm counts experienced during AP extract administration (the count per cc. was 2.2 times greater than any previous count) is probably indicative of gametogenic stimulation. Motility and morphology of the sperm were normal throughout the study.

No level of urinary androgens during gonadotropin administration was as high as the normal level of 47 I.U. encountered prior to treatment. Assays within

TABLE 8. SPERMATOGENIC RESPONSE TO GONADOTROPINS (*Case 15*)

Date	Treatment	Dosage	Count per cc.	Total count	Motility (relative)	Morphology (% normal)
		U				
7/18/39	Testosterone propionate	225 mg. (injection) 225 mg. (inunction) since 6/27/39	64,000,000	192,000,000	—	10
7/25/39	Testosterone propionate	75 mg. (injection) 75 mg. (inunction)	58,000,000	232,000,000	4+	10
9/26/39	Testosterone propionate	300 mg. (injection) 300 mg. (inunction)	44,000,000	88,000,000	3+	20
10/ 9/39	None	—	48,500,000	121,250,000	3+	18
12/ 2/39	None	—	108,000,000	540,000,000	3+ (50% alive)	15
12/ 9/39	APL	5,000	108,000,000	486,000,000	3+	13
12/16/39	APL	7,500	192,000,000	537,600,000	4+	16
12/23/39	APL	7,500	112,000,000	358,000,000	4+	11
12/29/39	APL	7,500	184,000,000	772,800,000	4+	11
1/ 8/40	APL	7,500	153,000,000	673,000,00	3+	11
1/20/40	PMS	4,000	166,000,000	564,400,000	4+	10
3/ 9/40	AP	16,800	104,000,000	416,000,000	3+	11
4/29/40	AP	22,800	416,000,000	1,747,200,000	3+ (50% alive)	15

hypogonadal range were found during the use of both APL and PMS. Unfortunately, no androgen assay was done at the time that the marked numerical increase in spermatozoa occurred during AP extract administration.

TABLE 8A. ANDROGENIC RESPONSE TO GONADOTROPINS (*Case 15*)

Date	Treatment	Dosage	Androgen assay
		U	I.U.
4/22/39	None	—	47
12/ 3/39	Testosterone propionate	none since 10/28/39	40
12/10/39	APL	7,500	35
12/17/39	APL	7,500	29
12/24/39	APL	7,500	19
12/30/39	APL	5,000	44
1/13/40	APL	9,000 to 1/8/40	11
	PMS	1,600 from 1/8/40 to 1/13/40	
2/17/40	PMS	9,600	33
3/ 4/40	PMS	4,800	16

RESULTS

In this series of fourteen cases a total of 97 assays for urinary androgens were made in 9 patients, and 166 semen examinations were made in 12 patients.[5] Spermatogenic response during the administration of APL was followed in 3 cases, and no stimulation was detected. In 11 cases observations were carried out on spermatogenesis during courses of therapy with PMS. Stimulation occurred in 3 cases. In 8 instances, semen examinations were made during the use of AP extract. Improvement was demonstrated in 3. The levels of urinary androgens were measured in 5 cases while APL was administered. An apparently significant increase occurred in 2 instances. In 8 cases where such levels were studied in response to PMS, improvement occurred in 3. In 7 cases in which AP extract was used, no significant increase in urinary androgen excretion was found.

An analysis of these cases from the standpoint of the final diagnosis or chief complaint is presented in table 9.

The following facts are also of importance in analyzing the results of gonadotropin administration. In 2 (*cases 4* and *13*) of the 12 cases where spermatogenesis was followed, the production of sperm was depressed by the previous use of testosterone propionate. In 2 cases (*cases 14* and *15*) the spermatozoa were numerically normal prior to the use of gonadotropins. In 6 (*cases 6, 7, 10, 13, 14,* and *15*) of the 9 cases where urinary androgen excretion was followed, the excretion levels were normal before gonadotropins were used. These facts undoubtedly had some bearing upon the response of the patients, and increased the difficulty of analyzing apparent increases in both spermatogenic and androgenic response.

No attempt has been made to analyze these cases from the standpoint of gonadotropin dosages, since

[5] We are indebted to the members of the research staff, particularly D. R. McCullagh, Ph.D., Wendell Bowman, Ph.D., and Mr. Ross Guillet for their cooperation in carrying out these laboratory procedures.

TABLE 9. SUMMARY OF RESULTS IN SPERMATOGENESIS AND ANDROGEN EXCRETION FOLLOWING TREATMENT WITH GONADOTROPINS

Gonadotropin	Pituitary Disease (4 cases)				Infertility (6 cases)				Infertility (X-ray) (1 case)				Impotence (2 cases)				Alopecia (1 case)			
	Androgenic		Spermatogenic		Androgenic		Spermatogenic		Androgenic		Spermatogenic		Androgenic		Spermatogenic		Androgenic		Spermatogenic	
	Tr.	Imp.	Tr.	Im.	Tr.	Imp.	Tr.	Imp.	Tr.	Imp.	Tr.	Imp.	Tr.	Imp.	Tr.	Imp.	Tr.	Imp.	Tr.	Imp.
APL	2	1	0	—	0	—	0	—	0	—	0	—	2	1	2	0	1	0	1	0
PMS	3	2	2	0	2	1	5	3	0	—	1	0	2	0	2	0	1	0	1	0
AP	2	0	1	1	3	0	3	1	0	—	1	0	2	0	2	0	0	—	1	1

the doses in most instances were as large or larger than those ordinarily employed for therapeutic purposes. It is possible that the larger dosages may have had an untoward rather than beneficial effect in some cases. The demonstration of an antigonadotropic substance in the serum of one patient while receiving AP extract is of interest in this connection.

The time intervals during which various gonadotropins were administered have varied widely and cannot be satisfactorily analyzed.

It is of interest that pregnancy did not result in any of the 8 cases in which infertility constituted the chief complaint. It was not possible, however, to examine the marital partner in all of these cases. The wife of *Case 6* has subsequently become pregnant following his receiving AP extract made from sheep pituitary.[6]

In general, our results demonstrate a wide variation in human spermatogenesis and androgen production as a result of gonadotropin administration. It is probably significant that no increase in spermatogenesis occurred during the administration of APL, and no increased urinary androgen excretion occurred while AP extract was used. It is interesting that in *case 5* (fig. 2) there is demonstrated a significant and consistent rise in urinary androgens following the use of PMS. This increase gradually disappeared while the patient was taking AP extract. The spermatogenic response was not associated in this case with any evidence of increased androgen production. In *case 6* (fig. 3) in which there was a spermatogenic effect from PMS, no increase in androgen production was apparent. Usually there has been no evidence of stimulation of the interstitial cells as judged by urinary androgens when a spermatogenic response occurred.

SUMMARY

Spermatogenic and androgenic responses to 3 different gonadotropins, APL, PMS, and AP extract were studied in a series of 14 patients, who had various clinical problems. Androgenic response, alone, was studied in 2 cases; spermatogenic response, alone, was studied in 5 cases; both responses were followed in 7 cases. Spermatogenesis was studied in 3 instances with APL, in 11 with PMS, and in 8 with AP extract. Androgenic response was followed in 5 cases with APL, in 8 cases with PMS, and in 7 cases with AP extract. A total of 97 assays for urinary androgens and 166 semen examinations were made.

The results of this survey were widely variable, and are summarized in table 9, where they are tabulated on the basis of the patient's chief complaints or final diagnoses.

[6] Gonadophysin, G. D. Searle & Co., Chicago, Ill.

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Endocrine Therapy of Functional Menometrorrhagia and Ovarian Sterility

III. The Cyclic Administration of Equine Gonadotropin[1]

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[Ovarian Sterility]

Gonadotropic therapy in anovulatory ovarian failure, in general, has been disappointing in its results. Of all the gonadotropins, the chorionic one of pregnancy urine has proven of least value. The consensus of most critical therapeutists is that it is incapable of inducing ovulation in this type of ovarian failure (1). An open minded attitude continues to prevail regarding the possible value of pituitary gonadrotropin although, as yet, extracts of pituitary gland containing this principle have not been available in such concentration and purity to warrant any serious therapeutic expectations. Equine gonadotropin, prepared from the serum of pregnant mares, despite the therapeutic expectations aroused by the clinical studies of Davis and Koff (2) which showed that augmentational phenomena were produced in normally responsive ovaries, has failed, in the opinion of many workers, to give good account of itself when used to treat the pathologic ovary. There has been considerable reliable clinical evidence that follicle stimulation and ripening and even striking cystic alterations may be produced in sensitive ovaries, but few critical data have been submitted that ovulation might be induced in ovaries not spontaneously exhibiting this function. The present communication, and the one to follow, attempt to evaluate the therapeutic efficiency of equine gonadotropin in anovulatory ovarian failure.

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Rationale of Gonadotropic Therapy

Gonadotropic therapy may be viewed as being justified on one of two grounds: *a*), that a patient's pituitary supplies inadequate gonadotropins to maintain efficient ovarian function; or *b*), that the ovaries are 'sluggish' or 'lazy' and need to be aroused by supplying additional stimuli. This latter premise is not in sympathy with present scientific concepts of tissue receptivity: ovaries which do not respond to normal levels of gonadotropins are not 'lazy' but intrinsically impaired in function, i.e., they have developed lowered receptivity or refractivity to the tropic influences of the pituitary.

The hope of success from attempts at gonadotropic therapy is based upon two assumptions: *a*), that ovaries, in instances of deficient pituitary function, retain their normal reactivities, which will permit them to give physiologic responses when adequate gonadotropic levels are assured; and *b*), that the extracts of gonadotropins available at present are capable of complementing or supplementing completely for pituitary deficits when these exist. Neither of these two assumptions may be accepted, at present, as having been proven definitely to be facts.

Ovarian receptivity. Pathologic considerations warrant the belief that diverse factors may cause functional ovarian failure. From one point of view functional ovarian failure may be classified as follows: *a*), that in which ovaries retain normal sensitiveness to gonadotropins, the cause of the failure being the fact that the pituitary has deficient function; *b*), that in which ovaries are only partially receptive to intrinsic pituitary influences, which in themselves are

normal, i.e., these ovaries give only partial responses of estrogenic nature, follicle maturation, with subsequent cystic degeneration in the absence of ovulation; and *c*), that in which ovaries are completely or almost completely refractive to intrinsic pituitary influences which are normal or greater than normal, i.e., minimal or no follicular activity occurs, as in a senile ovary.

There are numerous conditions which may alter the receptivity of ovaries: immaturity, senility and pregnancy; damage from roentgen-ray, radium or surgery; infection in or about the ovaries; constitutional debility; disturbed metabolism, i.e., decrease of thyroid or adrenal function; and irreparable degenerative changes following severe or long standing hypopituitarism.

Diagnosis of the degree of reactivity of the ovaries, at present, is usually indirect and is derived from two classes of data: *a*), the establishment by well controlled trials of gonadotropic therapy that hypofunctioning ovaries do or do not give full normal responses during treatment; and *b*), proof of the existence of inadequate pituitary function as the cause of ovarian failure may warrant tentative assumptions of normal ovarian receptivity. The methods for the diagnosis, however, of levels of pituitary function are not satisfactory at present, especially those which seek to establish gonadotropic titers of body fluids. The other data constitute little more than a means for clinical inferences. Were it possible to establish with certainty the existence and the degree of pituitary deficits, these facts would not necessarily prove that the ovaries under these circumstances could be depended upon to respond to adequate gonadotropic therapy. Animal experiments have shown that the recentness or remoteness of the initiation of therapy in regards to the time of hypophysectomy conditions the responses to therapy in hypophysectomized animals; irreparable gonadal degenerations may result if therapy is postponed too long (3, 4). Similar phenomena may occur clinically if severe hypopituitarism exists. Under such circumstances, it might be that gonadotropic therapy is capable of salvaging only those patients with moderate pituitary impairment.

Effectiveness of available gonadotropins in complemental or substitutional therapy of pituitary failure. The present concept is that gonadotropic therapy, while it may prove stimulative as far as the ovaries are concerned, is purely substitutional as regards the pituitary failure, i.e., substitution at the pituitary level with gonadotropins is similar in kind to steroid therapy which comprises substitution at the ovarian level with estrogen and progesterone. This being true, like all substitution therapy, gonadotropic therapy would be expected to be effective only so long as it were continued. The fact that the preparations of gonadotropins available at present contain proteins or protein-derivatives, which have been shown to produce antibody reactions if treatments be continued for long periods (5), renders continued substitution at the pituitary level impossible.

Either chorionic gonadotropin or the presently available preparations of pituitary gonadotropins has not been shown to be capable of providing effective substitutional therapy for pituitary failure. Some evidence has been submitted that a combination of pituitary extracts containing little gonadotropic activity with chorionic gonadotropin results in augmentational or synergistic phenomena, which permit the production of striking hemorrhagic and lutein alterations in the ovaries of patients treated. A recent report upon the therapeutic use of such a preparation (not yet available commercially) presents some encouraging results (6).

Equine gonadotropin is considered by some workers to be a 'complete' gonadotropic hormone, possessing both follicle-stimulating and luteinizing properties (7). It has been reported capable of substituting completely for the gonad-stimulating functions of the anterior lobe of the pituitary gland in some half dozen species (8). Evidence that this gonadotropin fulfills all the substitutional requirements for gonad stimulation in the monkey and man is lacking. Hartman (9) was unable to induce ovulation except, perhaps, in a few isolated instances in monkeys with ovarian sterility by intravenous injections of equine gonadotropins, in amounts similar to those employed by Davis and Koff in women. Smith (10) reported this gonadotropin incapable of preventing or repairing posthypophysectomy damage in the gonads of male monkeys. We have reported on a number of occasions (1, 11, 12) that we had been unable to obtain any consistent data to the effect that ovarian failure of adult women could be corrected and normal ovarian responses induced by therapy with equine gonadotropins, although some striking therapeutic salvages had been obtained by us in a few patients with adolescent ovarian failure (13). Erving, Sears and Rock (14) have reported recently similar experiences. The present communication presents our therapeutic data.

In view of the fact that Engle and Hamburger (15) had been able to induce ovulation in the monkey only by the combined and sequential use of pituitary and chorionic gonadotropins, and since Büttner (16) had reported some degree of success from similar therapy given to women, we began a study of the gonadotropic effects produced by the one-two administration of equine and chorionic gonadotropins. It was our purpose to investigate the possibility that a synergism might exist between these two gonadotropins which might be necessary to secure a complete pituitary substitutional effect. We likened substitution at the pituitary level with equine gonadotropin followed by

chorionic gonadotropin to substitution at the ovarian level with estrogen followed by progesterone, a well known one-two synergism. Previous reports by one of us (12, 13) have indicated the occurrence of unequivocal instances of therapeutic salvage from this form of therapy. The next communication of this series will report these therapeutic data and compare them with those obtained in the present study in which only equine gonadotropin was employed.

Therapeutic Regime

The regime employed in the present study was as follows. All patients were skin tested for possible allergy prior to the initiation of any therapy. Patients who had taken one series of therapy without allergic manifestations, were reinvestigated, however, before additional therapy was given. The intramuscular route of administration was employed in all but several instances when the gonadotropin was given intravenously. (These exceptions will be cited specifically when they are encountered in the data.)

The administration of the gonadotropin has been carried out in a cyclic manner except in a few instances, which will be considered subsequently as exceptions. Except in instances wherein long intervals existed between episodes of bleeding, treatment was started promptly at the conclusion of bleeding. Injections were given daily for approximately 10 days, whereby the duration of therapy was comparable to that of the phase of follicular maturation. No further therapy was given until adequate time had elapsed for the corpus luteum phase of the cycle, in the event ovulation had been induced. If bleeding occurred at or about the usual expected time, about 14 day after the last injection, no further therapy was given until bleeding ceased and then, if clinical considerations warranted it, another series of therapy similar to the first one was given. If no bleeding followed therapy, another series of therapy was started within 14 to 16 days after the cessation of the first series, so that cyclicity of the treatment was not disturbed. In no instances were more than 2 series of therapy given without rest periods of at least 1 month intervening; this routine was out of respect for the possible development of antibodies. The usual daily doses of equine gonadotropin ranged from 200 to 800 I.U., the usual daily dose was 400 I.U.

METHOD OF STUDY

The basis for the evaluation of gonadotropic responses in this study was the endometrial responses at the conclusion of therapy or at the onset of episodes of flowing following therapy. These endometrial data became significant when compared with the findings in the endometria at the onset of several or more flowings of each patient prior to the initiation of treatment. Only those women whose pretreatment data indicated with a fair degree of certainty that their bleedings habitually occurred from estrogenic endometria (i.e., were of anovulatory nature) were chosen for the assessment of gonadotropic therapy. The finding of a progestational endometrium at the conclusion of therapy, or the occurrence of a pregnancy during or immediately following therapy was taken to be evidence of a specific gonadotropic effect. In addition to these endometrial data, which were considered of primary importance, observations were made also on the cyclicity and duration of bleeding during and following therapy; these data were considered of secondary importance in the present study.

Endometrial specimens were considered significant for evaluation under the following conditions. All pretreatment endometrial data were based upon biopsies taken within the first 12 to 18 hours after the onset of spontaneous episodes of bleeding. At least 2 consecutive episodes of estrogenic bleeding prior to the initiation of therapy were required to warrant the assumption that this type of bleeding was characteristic of the patient. When patients had required curettage as a method of hemostasis in menorrhagia or metrorrhagia, the endometrial findings on curettage were not accepted but, instead, the endometrial findings at flowings subsequent to curettage were used as pretreatment data. In the instance of patients who had had previous therapies, at least one nontreatment cycle was allowed to elapse before starting new therapy. Endometrial biopsies were made at the onset of flowings which followed cycles of therapy. If no flowing followed therapy, a biopsy was made, nevertheless, within 14–16 days after the last injection of therapy had been given (i.e., after sufficient time had elapsed for a progestational reaction of the endometrium had ovulation and corpus luteum activity been inaugurated by therapy). Following discontinuation of cycles of therapy, the endometrial responses at the onset of flowing which had not been preceded by therapy were determined in order to establish the possible permanency of any alterations in ovarian function produced by therapy.

All patients studied had had complete medical, gynecologic and endocrine surveys which, as a minimum requirement, included determinations of basal metabolic rates and roentgenographic investigations of the sella turcica. In a number of patients, pretreatment and treatment levels of the excretion of sodium pregnandiol glucuronide (determined by Venning's method, 17) and of 17-ketosteroids (estimated by Oesting's method, 18) were established. These data are reported as being of possible significance in the present study.

Some patients, other than those with estrogenic bleeding whose endometrial responses to cyclic

TABLE 1. COMPOSITE CLINICAL DATA OF 23 PATIENTS TREATED BY EQUINE GONADOTROPIN GIVEN BY INJECTIONS

Case No.	Age	Menstrual History	Endometrium Before Treatment	Nature of Treatment and Endometrial Responses during Therapy	Endometrium After Treatment	Menstrual History after Therapy and Follow-up Data
				Group 1		
EG13	23	Mm	E+	E+(3600)[1]	θ	Amenorrhea 4 mo.→CySt
EG30	31	Mm	E	E(2800)37 θ(4400)	E	Amenorrhea 2 mo. No return
EG31	14	Mm	E	E(2800)34 E(4400)24	E	Regular. OP 4 mo.
EG39	17	Mm	E	E(4400)24 E(4400)33	O	→CySt
EG40	19	Mm	E+	E(4400)36	O	Menorrhagia 2 mo.→CySt
B.4	14	Mm	E+	E(4000)40 E+(4000)23	O	Menorrhagia 21 days→CySt
O33	14	Mm	E	E(4400)37[2]	O	Menorrhagia 2 mo. No return
B.9	26	Mm	E	E(2600)39 E(2800)31	M	Regular 1 mo.→CySt
EG12	26	Ol	E−	E−(4000)17[3]	E	Menorrhagia 4 mo.→CySt
EG32	27	Ol	E	Eθ(2600)	—	No data
EG33	22	Ol	E−	Eθ(4800) Eθ(5600)	Eθ	Amenorrhea 2 mo.→CySt
EG34	30	Ol	E	E+(2800)22	O	Regular.→CySt
EG35	27	Ol	E−	M(5600) E(4000)48	O	Amenorrhea 3 mo.→CyG
EG36	29	Ol	E−	Eθ(14600 M.U.)[4]	θ	Amenorrhea. No return
EG37	19	Ol	E	Eθ(4000)	θ	Amenorrhea 1 mo.→CySt
EG38	19	Ol	E	E+(2800)35 E(4000)48	O	→CySt
I	31	Ol	E−	E−θ(4200 M.U.)	θ	Amenorrhea 1 mo. No return
				Group 2		
EG45	26	Ol	E	θ(4000) θ(4000)	Eθ	→CyE
EG46	15	Ol	E	θ(5600) θ(5600)	θ	No data
EG53	35	CyS	M	O(2400) M(2400)	M	→CySt
EG54	18	CyS	M	O(4400)	O	No return
EG55	30	CyS	M	O(8250) O(2400)	O	OP 3 mo.
EG44	40	CyS	—	O(5250) O(6750)	O	OP

[1] Last injection (800 I.U.) was given intravenously. Treatment was given during an episode of bleeding which became more profuse and required a curettage for hemostasis.
[2] Treatment given during spotting which lasted for the first 21 days of the cycle.
[3] Last injection (800 I.U.) was given intravenously.
[4] Treatment covered a period of 53 days.
Menstrual history: Mm, menometrorrhagia; Ol, oligomenorrhea; CyS, cyclic bleeding sterility. Endometrium at bleeding (or at time when bleeding should have occurred) before, during and after treatment; E−, hypoestrogenic endometrium; E, persistent estrogenic endometrium; E+, hyperestrogenic endometrium; M, mixed progestational endometrium; O, bleeding occurred but no biopsy was taken; θ, no bleeding occurred after a period of treatment. The numerals in parentheses following endometrial responses signify the number of international units (except in 2 instances where mouse units are indicated) of equine gonadotropin administered during that period. Numerals after parentheses signify length of cycle; the absence of numerals signifies length of cycle was within normal range, i.e., 25–30 days. Follow-up data; CySt indicates that the patient was transferred to cyclic steroid therapy administered by injection; CyG indicates that patient was transferred to cyclic gonadotropic therapy; CyE indicates that the patient was transferred to cyclic estrogenic therapy given by injection; OP followed by the number of months indicates that no further treatment has been given during the specified interval of months.

gonadotropic therapy were studied, are included for evaluation of the effects of therapy upon the cyclicity of bleeding or upon the urinary hormonal titers or for the assessment of the sex-maturing effects of equine gonadotropins in adolescent hypoövarianism. The varying therapeutic schedules of these patients will be cited briefly in their protocols.

CLINICAL DATA

A total of 31 women, whose ages ranged from 14 to 40 years, was studied. These women were divided into 3 groups depending upon the clinical findings and symptoms as well as upon the nature of the observations permitted:

Group 1 was comprised of 17 women whose ages ranged from 17 to 31 years. These women presented oligomenorrhea (9 cases) or menometrorrhagia (8 cases) as symptoms of ovarian failure. In all of these women bleeding occurred from estrogenic endometria. Assessments of gonadotropic effects upon the basis of endometrial responses and effects upon acyclic bleeding were made in all 17 instances.

Group 2 was comprised of 6 women whose ages ranged from 15 to 40 years. These women presented oligomenorrhea (2 cases) and ovarian sterility associated with cyclic bleeding (4 cases) as symptoms of ovarian failure. In the 2 patients with oligomenorrhea, bleeding was of estrogenic nature; in 3 of the 4 patients with presumed ovarian sterility, it was associated with endometria which showed irregular and incomplete progestational responses and in the 4th patient endometrial studies were not made. Data in regard to the endometrial responses to therapy of these patients are not significant. The 6 patients of

TABLE 2. CLINICAL DATA ON 8 PATIENTS SHOWING MINIMAL OR INCOMPLETE SEXUAL MATURATION

Case No.	Pretreatment Data	Treatment	Post-Treatment Data
EG47	Aged 16 years; delayed menarche; rudimentary uterus; sexual maturation normal otherwise. No endometrial studies.	375 I.U. equine gonadotropin q2d for 6 weeks, total 7,120 I.U.	No enlargement of ovaries or uterus. No bleeding. No endometrial studies.
B5	Aged 22 years: delayed menarche; juvenile uterus and cervix, ⅔ normal size; ovaries not palpable; moderately hypoplastic breasts; dwarfed stature; osseous age 14 years. Hypoestrogenic endometrium	400 I.U. equine gonadotropin daily for 6 weeks, total 16,400 I.U. Rest period for several weeks, then 800 I.U. for 3 weeks, total 16,000 I.U.	No enlargement of uterus; ovaries not palpable. No bleeding. Hypoestrogenic endometrium.
EG42	Aged 25 years: long episodes of amenorrhea since menarche at 16; no bleeding for 11 months prior to R_f; uterus and cervix juvenile type and ⅔ normal size; vagina and external genitalia moderately hypoplastic; scanty pubic hair; hypoplastic breasts; dwarfed stature. Estrogenic endometrium.	400 I.U. equine gonadotropin, q2d for 5 weeks, total 6,200 I.U.	No enlargement of uterus or ovaries. Episode of bleeding followed R_f; endometrium, persistent estrogenic.
EG48	Aged 18 years: amenorrhea of 2 years' duration, preceded by oligomenorrhea since menarche; uterus and cervix, juvenile type, ½ normal size; ovaries ½ normal size; sexual maturation of early adolescent type. No endometrial studies.	400 I.U. equine gonadotropin q2d for 6 weeks, total 8,400 I.U.	No enlargement of uterus or ovaries. No bleeding. No endometrial studies.
EG41	Aged 24 years: delayed menarche; uterus and cervix extremely rudimentary and not identified as such; ovaries not palpable; moderate hypoplasia of vagina and external genitals; hypoplastic breasts; scanty pubic hair; osseous age, 15 years. No endometrial studies.	200 I.U. equine gonadotropin daily for 18 days, total 3,600 I.U. Rest period for 6 weeks, then 200 I.U. q2d for 6 weeks, total 3,200 I.U.	No enlargement of uterus or ovaries. No bleeding. No endometrial studies.
EG49	Aged 26 years: delayed menarche; uterus and cervix markedly hypoplastic; ovaries not felt; external genitals and vagina slightly hypoplastic; juvenile breasts; long legs and arms; osseous age 16 years. No endometrial studies.	4 series of R_f with rest period of 2–6 weeks intervening. 375 I.U. equine gonadotropin q2d for 8 weeks, total 9,375 I.U. 750 I.U. equine gonadotropin q2d for 4 weeks, total 9,000 I.U. 400 I.U. equine gonadotropin daily for 14 days, total 5,600 I.U. Another series, same as the previous one.	No enlargement of uterus or ovaries. No bleeding. No endometrial studies.
EG50	Aged 17 years: delayed menarche; juvenile uterus and cervix, ⅓ normal size; ovaries not felt; hypoplasia of vagina and external genitals; scanty axillary and pubic crines; long arms and legs; moderately hypoplastic breasts; osseous age, 14 years. No endometrial studies.	100 M.U. equine gonadotropin q2d for 20 days, total 1,000 M.U. Rest period of 4 weeks, then 200 M.U. equine gonadotropin q2d for 6 weeks, total 3,400 M.U.	Vagina and uterus enlarged. Left ovary palpable. Endometrial biopsy; uterus 2 inches in depth. Estrogenic endometrium. Uterus enlarged to 2½ inches in depth. Biopsy at conclusion of R_f: progestational endometrium. First menstrual period 2 days after biopsy. Subsequent menses regular and associated with normal ovarian function.
EG51	Aged 19 years: delayed menarche; uterus and cervix, juvenile, not over 1 inch in size; ovaries not felt; hypoplastic vagina and external genitals; juvenile breasts; long legs and arms; osseous age, 16 years. No endometrial studies.	3 series of R_f with rest periods of 6–8 weeks intervening. 200 I.U. equine gonadotropin q2d for 6 weeks, total 3,600 I.U. 375 I.U. equine gonadotropin daily for 5 weeks, total 13,500 I.U. 750 I.U. equine gonadotropin q2d for 5 weeks, total 13,500 I.U.	No enlargement of uterus or ovaries. No bleeding. No endometrial studies.

this group are added to those of group 1 for assessment only of the effects of therapy on acyclic bleeding.

The pertinent clinical data on patients of *groups 1* and *2* are presented in table 1. All these patients received cyclic gonadotropic therapy.

Group 3 was comprised of 8 women, whose ages ranged from 16 to 26 years. The common finding in these was adolescent hypoövarianism which was characterized by diverse grades of impairment of sexual maturation. The majority of these patients had never experienced menarche; the remainder were experiencing long episodes of amenorrhea. The evaluation of gonadotropic therapy in these patients was based on: *a*), clinical evidence of increased estrogenic

output of ovaries, i.e., increased sexual maturation; and *b*), induction of uterine bleeding. The clinical data on these patients are presented in the form of brief individual protocols in table 2.

Endometrial responses of group 1. The endometrial findings of the 17 patients during pretreatment studies were distributed as follows:

Hypoestrogenic (E −), 5 patients
Persistent Estrogenic (E), 9 patients
Hyperestrogenic (E +), 3 patients

A total of 25 cycles of therapy was given these patients, details of each cycle of which are given in table 1. The average amount of equine gonadotropin given per cycle (exclusive of 2 cycles in which a Danish preparation was used, the units of which were not transferable to I.U.) was 3,878 I.U. The endometria were sampled at the conclusion of 24 cycles of the 17 patients in which therapy was given. The endometrial findings were as follows:

Hypoestrogenic (E −), 2 occurrences
Persistent estrogenic (E), 17 occurrences
Hyperestrogenic (E +), 4 occurrences
Mixed progestational (M), 1 occurrence (i.e., 4.16% incidence)

As regards individual patients, only one of the 17 patients gave a positive (progestational) response during the treatment period.

The follow-up records of these patients after one or more cycles of no therapy were:

No bleeding and no biopsies made, 4 patients
No bleeding but a biopsy made, 1 patient
Bleeding occurred but no biopsies made, 7 patients
Bleeding occurred and biopsies made, 4 patients
No data, 1 patient

The endometrial findings on the 5 patients who had follow-up biopsies were:

Persistent estrogenic (E), 4 patients
Mixed progestational (M), 1 patient

Alterations in the character of uterine bleeding (groups 1 and 2). The effects of therapy upon the existing irregularities of bleeding (oligomenorrhea and menometrorrhagia) or upon cyclic bleeding were investigated in the 23 patients of *groups* 1 and 2. The immediate effects of each of the 16 cycles of therapy given upon the oligomenorrhea of 11 of these patients were:

Bleeding occurred at the calculated time as regards cyclicity of therapy, 1 instance
Oligomenorrhea not changed, 3 instances
Polymenorrhea occurred, 2 instances
No bleeding occurred immediately following therapy (cycles longer than 50 days), 10 instances

The immediate effects of each of the 13 cycles of therapy given upon the menometrorrhagia of 8 of these patients were:

Bleeding occurred at the calculated time as regards cyclicity of therapy, 1 instance
Oligomenorrhea occurred, 7 instances
Polymenorrhea occurred, 3 instances
No bleeding occurred immediately following therapy (cycles longer than 50 days), 1 instance
Therapy given during bleeding episode, the severity of which was aggravated and curettage was necessary for hemostasis, 1 instance

Therapy had no effects on the cyclic bleeding of 4 patients who received 7 series of treatments.

The average durations of episodes of bleeding which immediately followed series of therapy were 4.5 days for the oligomenorrhea group and 14.5 days for menometrorrhagia (with exception of 1 indeterminate episode of bleeding which required curettage for hemostasis).

The follow-up data upon the bleeding histories of the patients of the oligomenorrhea and menometrorrhagia groups are summarized below. Of the 11 patients with oligomenorrhea,

1 developed menorrhagia;
5 had episodes of amenorrhea of varying durations;
1 had cyclic bleeding;
2 gave no follow-up data;
2 received additional therapy before sufficient time had elapsed for ultimate evaluation of the cyclic gonadotropic therapy.

The majority of these patients were transferred subsequently to other therapeutic schedules, the nature of which is indicated in table 1.

Alterations in urinary titers of pregnandiol-complex and 17-ketosteroids. The effects of therapy on the urinary excretion of sodium pregnandiol glucuronide and of 17-ketosteroids were available for study in cases of 2 patients of *group* 1 and 3 patients of *group* 3. These data are given in table 3.

TABLE 3. EFFECTS OF CYCLIC EQUINE GONADOTROPIN THERAPY UPON THE URINARY EXCRETION OF SODIUM PREGNANDIOL GLUCURONIDE AND OF 17-KETOSTEROIDS OF 5 PATIENTS

Case No.	Total Sodium Pregnandiol Glucuronide Excreted during a Non-bleeding Cycle		Av. Daily Excretion of 17-Ketosteroids during a Non-bleeding Cycle	
	Before ℞	During ℞ Cycle	Before ℞	During ℞ Cycle
	mg.	mg.	I.U.	I.U.
EG36	17	44		
EG41	0	0	22	33
EG42	0	3	30	29
B5	0	0		
EG44	0	4	43	58

Effects on delayed or incomplete sexual maturation.

In the instances of 8 patients of group 3, we investigated the effectiveness of equine gonadotropin, given as a rule in series of 6 weeks' duration with rest periods intervening, with regards to the initiation or enhancement of sexual maturation. These clinical data are summarized in table 2. In only one instance, did any specific responses occur and here they were striking. (The details of this patient's (EG 50) response have been reported in extenso by one of us (11). Detailed reports of the responses of 3 other patients of this group have also been made (13).)

DISCUSSION

These data do not indicate the occurrence of any significant therapeutic salvage from the use of equine gonadotropins in the dosages and according to the schedules of administration employed by us.

Data of diverse sorts, i.e., endometrial responses, effects on irregularities of bleeding and alterations in urinary titers of the pregnandiol-complex, point preponderantly to the non-production of any specific gonadotropic effects. The single occurrence of a progestational response in the endometrium of one of the 17 patients in *group 1* with estrogenic bleeding may perhaps have resulted from a spontaneous remission of ovarian failure.

It may be regarded as possible, but it is highly improbable, that these 17 patients all had ovaries which were refractive to gonadotropins, i.e., the ovarian failure was of intrinsic origin. These 17 patients, however, were selected for therapy in the same random manner as those, the results of whose treatment with one-two cyclic gonadotropic therapy (i.e., equine followed by chorionic gonadotropins) are reported in the next communication of this series. Anticipating somewhat the data of the latter study, it may be stated that the results of the combined therapy were far from unfavorable as regards endometrial responses. As a matter of fact, 2 of the patients of the present study, i.e., *cases* 033 and *B5*, who gave negative endometrial responses to equine gonadotropic therapy subsequently had responded to one-two gonadotropic therapy.

The data reported indicate that cyclic equine gonadotropic therapy offers no significant aid in the regulation of irregular uterine bleeding such as that which characterized the cyclic use of steroids (19). The findings in this study and the succeeding one show that no clear-cut correlation exists between the regulation of bleeding and the production of specific endometrial responses. Further discussion of this fact will be presented in the next communication.

The one striking salvage, which was obtained from the 8 patients of *group 3* with incomplete or absent sexual maturation, is relatable to the existence of pituitary deficiency of such a minimal nature that the pituitary was able to augment by its own activity the equine gonadotropin administered. The subsequent clinical record of the patient tends to bear out this assumption: once bleeding was initiated, it continued cyclicly and normally as regards ovarian responses without any further recourse to therapy being necessary, i.e., no additional complementing or supplementing for pituitary failure was necessary.

SUMMARY

The responses of 31 women, aged 14 to 44 years, to therapy with equine gonadotropin were studied. Only one progestational response occurred in the endometrium of one of 17 women with estrogenic bleeding treated with 25 series of equine gonadotropin, given, as a rule, in daily intramuscular doses of 400 I.U. for 10 consecutive days following cessation of bleeding. Observations upon the bleeding cycles and durations of flowing of these 17 women and an additional group of 5 others did not indicate any salutary regulation of these from therapy. Studies of the pregnandiol-complex excretion before and during treatment of the cycles of 5 women revealed no significant alterations in these. Eight patients with absence of, or incomplete sexual maturation were treated with equine gonadotropin. In only one case were any specific effects produced; these were characterized by a complete salvage of ovarian function.

Equine gonadotropin for these studies was supplied by Schering Corporation, Bloomfield, N. J., (Anteron), The Upjohn Company, Kalamazoo, Mich., (Gonadogen), Cutter Laboratories, Berkeley, Calif., (Gonadin) and by Ayerst, McKenna & Harrison, Montreal, Canada, (Antex, Leo).

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Endocrine Therapy of Functional Menometrorrhagia and Ovarian Sterility

IV. One-Two Cyclic Therapy with Equine and Chorionic Gonadotropins[1]

E. C. Hamblen, M.D., W. Kenneth Cuyler, Ph.D., J. A. Wilson, M.D., and R. L. Pullen, M.D.

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In view of the lack of evidence, secured from the studies of the preceding communication, that the cyclic administration of equine gonadotropins affords any significant salvage in anovulatory ovarian failure, and because the theoretical and experimental considerations reviewed previously indicated that in the cases of the monkey and woman 'augmentation' or 'synergism' of equine gonadotropin with chorionic gonadotropin was doubtlessly necessary for full supplementation of pituitary deficiency, the present study was made. It is concerned with the assessment of effectiveness of a system of one-two cyclic therapy with equine and chorionic gonadotropins. The rationale of this form of therapy has been considered in detail in the preceding communication.

Therapeutic Regime

The same general routine, which was described in the previous communication, was followed in the present study with the modification imposed by adding 10 days of chorionic gonadotropic therapy to the 10 days of equine gonadotropic therapy employed therein.

The same precautions were observed in regard to the skin testing of patients for possible allergy. All therapy was given by the intramuscular route. Cyclicity of administration was observed in the same manner hitherto delineated. If no bleeding occurred within 4 to 6 days after the cessation of therapy, another series of treatment was started. No more than 2 series of this therapy were given in succession lest untoward responses from antibody formation result. At least one non-treatment cycle was allowed to elapse before additional therapy was given.

The schedule for a series of therapy was essentially as follows. Immediately at the conclusion of an episode of bleeding, equine gonadotropic therapy was begun and an intramuscular injection was given daily for 10 consecutive days; directly following this part of the treatment, chorionic gonadotropic therapy was started and an intramuscular injection was given daily for the next 10 consecutive days. If bleeding started before therapy was completed, injections were discontinued lest flowing be exaggerated or treatment get 'out of step' with the cycle of bleeding. The daily doses of equine gonadotropin varied from 200 to 800 I.U., the usual daily dose being 400 I.U. The daily doses of chorionic gonadotropin varied from 300 to 500 I.U., the usual daily dose being 300 I.U.

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In the instances of 8 patients of group 3, we investigated the effectiveness of equine gonadotropin, given as a rule in series of 6 weeks' duration with rest periods intervening, with regards to the initiation or enhancement of sexual maturation. These clinical data are summarized in table 2. In only one instance, did any specific responses occur and here they were striking. (The details of this patient's (EG 50) response have been reported in extenso by one of us (11). Detailed reports of the responses of 3 other patients of this group have also been made (13).)

DISCUSSION

These data do not indicate the occurrence of any significant therapeutic salvage from the use of equine gonadotropins in the dosages and according to the schedules of administration employed by us.

Data of diverse sorts, i.e., endometrial responses, effects on irregularities of bleeding and alterations in urinary titers of the pregnandiol-complex, point preponderantly to the non-production of any specific gonadotropic effects. The single occurrence of a progestational response in the endometrium of one of the 17 patients in *group 1* with estrogenic bleeding may perhaps have resulted from a spontaneous remission of ovarian failure.

It may be regarded as possible, but it is highly improbable, that these 17 patients all had ovaries which were refractive to gonadotropins, i.e., the ovarian failure was of intrinsic origin. These 17 patients, however, were selected for therapy in the same random manner as those, the results of whose treatment with one-two cyclic gonadotropic therapy (i.e., equine followed by chorionic gonadotropins) are reported in the next communication of this series. Anticipating somewhat the data of the latter study, it may be stated that the results of the combined therapy were far from unfavorable as regards endometrial responses. As a matter of fact, 2 of the patients of the present study, i.e., *cases* 033 and B_5, who gave negative endometrial responses to equine gonadotropic therapy subsequently had responded to one-two gonadotropic therapy.

The data reported indicate that cyclic equine gonadotropic therapy offers no significant aid in the regulation of irregular uterine bleeding such as that which characterized the cyclic use of steroids (19). The findings in this study and the succeeding one show that no clear-cut correlation exists between the regulation of bleeding and the production of specific endometrial responses. Further discussion of this fact will be presented in the next communication.

The one striking salvage, which was obtained from the 8 patients of *group 3* with incomplete or absent sexual maturation, is relatable to the existence of pituitary deficiency of such a minimal nature that the pituitary was able to augment by its own activity the equine gonadotropin administered. The subsequent clinical record of the patient tends to bear out this assumption: once bleeding was initiated, it continued cyclicly and normally as regards ovarian responses without any further recourse to therapy being necessary, i.e., no additional complementing or supplementing for pituitary failure was necessary.

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The responses of 31 women, aged 14 to 44 years, to therapy with equine gonadotropin were studied. Only one progestational response occurred in the endometrium of one of 17 women with estrogenic bleeding treated with 25 series of equine gonadotropin, given, as a rule, in daily intramuscular doses of 400 I.U. for 10 consecutive days following cessation of bleeding. Observations upon the bleeding cycles and durations of flowing of these 17 women and an additional group of 5 others did not indicate any salutary regulation of these from therapy. Studies of the pregnandiol-complex excretion before and during treatment of the cycles of 5 women revealed no significant alterations in these. Eight patients with absence of, or incomplete sexual maturation were treated with equine gonadotropin. In only one case were any specific effects produced; these were characterized by a complete salvage of ovarian function.

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IV. One-Two Cyclic Therapy with Equine and Chorionic Gonadotropins[1]

[Ovarian Sterility]

E. C. Hamblen, M.D., W. Kenneth Cuyler, Ph.D., J. A. Wilson, M.D., and R. L. Pullen, M.D.

From the Endocrine Division of the Department of Obstetrics and Gynecology, Duke University School of Medicine and Hospital, Durham, North Carolina

In view of the lack of evidence, secured from the studies of the preceding communication, that the cyclic administration of equine gonadotropins affords any significant salvage in anovulatory ovarian failure, and because the theoretical and experimental considerations reviewed previously indicated that in the cases of the monkey and woman 'augmentation' or 'synergism' of equine gonadotropin with chorionic gonadotropin was doubtlessly necessary for full supplementation of pituitary deficiency, the present study was made. It is concerned with the assessment of effectiveness of a system of one-two cyclic therapy with equine and chorionic gonadotropins. The rationale of this form of therapy has been considered in detail in the preceding communication.

Therapeutic Regime

The same general routine, which was described in the previous communication, was followed in the present study with the modification imposed by adding 10 days of chorionic gonadotropic therapy to the 10 days of equine gonadotropic therapy employed therein.

The same precautions were observed in regard to the skin testing of patients for possible allergy. All therapy was given by the intramuscular route. Cyclicity of administration was observed in the same manner hitherto delineated. If no bleeding occurred within 4 to 6 days after the cessation of therapy, another series of treatment was started. No more than 2 series of this therapy were given in succession lest untoward responses from antibody formation result. At least one non-treatment cycle was allowed to elapse before additional therapy was given.

The schedule for a series of therapy was essentially as follows. Immediately at the conclusion of an episode of bleeding, equine gonadotropic therapy was begun and an intramuscular injection was given daily for 10 consecutive days; directly following this part of the treatment, chorionic gonadotropic therapy was started and an intramuscular injection was given daily for the next 10 consecutive days. If bleeding started before therapy was completed, injections were discontinued lest flowing be exaggerated or treatment get 'out of step' with the cycle of bleeding. The daily doses of equine gonadotropin varied from 200 to 800 I.U., the usual daily dose being 400 I.U. The daily doses of chorionic gonadotropin varied from 300 to 500 I.U., the usual daily dose being 300 I.U.

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METHODS OF STUDY

Essentially the same criteria accepted for the evaluation of therapeutic results in the previous communication were employed in this study. The primary observations concerned endometrial data, the selection of which was done under the same rules of qualification hitherto stated. The finding of a progestational endometrium at the conclusion of a series of therapy in a woman previously experiencing only estrogenic endometrial function or the occurrence of a pregnancy during or immediately following therapy in a woman with a similar pretreatment level of ovarian function was taken to be evidence of a specific gonadotropic effect. Observations upon the permanency of gonadotropic effects produced by therapy were made up on the basis of endometrial studies made after the elapse of at least one non-treatment cycle. All endometrial biopsies were made usually within the first 12 to 18 hours after the beginning of uterine flowing. If no flowing occurred, however, within 4 to 6 days after a series of therapy had been concluded, a biopsy was made lest significant endometrial responses might have occurred without the manifestation of bleeding.

A secondary evaluation of the effectiveness of therapy was based upon regulatory effects exerted upon the irregular cycles of the patients with oligomenorrhea and menometrorrhagia.

All the patients studied had had complete medical, gynecologic and endocrine surveys which, as a minimum requirement, included determinations of basal metabolic rates and roentgenographic investigations of the sella turcica.

CLINICAL DATA

A total of 31 women, whose ages ranged from 14 to 36 years, was studied. All these women experienced bleeding from estrogenic endometria. The menstrual histories of these patients permit their being grouped into three categories: *a*) 3 women, aged 28 to 36 years, with cyclic bleeding (ovarian sterility was the reason for therapy); *b*), 19 women, aged 14 to 31 years, with menometrorrhagia; and *c*), 9 women, aged 16 to 29 years, with oligomenorrhea. The pertinent clinical data on these 31 patients are summarized in table 1.

Only 29 of the 31 women permit evaluation of endometrial responses to therapy since in one of the oligomenorrhea group (*case EG+CG15*) and in one of the menometrorrhagia group (*case O10*) no endometrial studies during treatment cycles were available. The records of these patients are included solely for studies of the effects of therapy upon the irregular bleeding cycles.

Endometrial responses. The pretreatment endometrial findings of the 29 patients to be evaluated on this basis were distributed as follows:

Hypoestrogenic (E −), 2 patients
Persistent Estrogenic (E), 24 patients
Hyperestrogenic (E +), 3 patients

A total of 57 cycles of therapy was given these 29 patients, details of each cycle of which are given in table 1. The average amount of equine gonadotropin given per cycle of therapy was 3,330 I.U.; the average amount of chorionic gonadotropin given per cycle of therapy was 3,215 I.U.

The endometrial responses were sampled by biopsies at the conclusion of 45 of the 57 cycles of therapy given the 29 patients. The findings on these biopsies were:

Hypoestrogenic (E −)	2 occurrences	62.2%
Persistent Estrogenic (E)	23 occurrences	
Hyperestrogenic (E +)	3 occurrences	
Mixed Progestational (M)	15 occurrences	37.8%
Normal Progestational (P)[1]	2 occurrences	

[1] One of these proved to have been associated with a pregnancy.

When individual patients were considered, it was found that 15 or 51.7% of the 29 patients yielded one or more progestational responses to therapy. The distribution of these positively responding patients according to their menstrual histories was:

Those with cyclic estrogenic bleeding, 3[1] of the 3 patients studied (100%)
Those with menometrorrhagia, 7 of the 18 patients studied (38.9%)
Those with oligomenorrhea, 5[2] of the 8 patients studied (62.5%)

[1] Includes 2 pregnancies.
[2] Includes 1 pregnancy.

The follow-up endometrial records of 17 of these 29 patients after one or more no-treatment cycles were:

Persistent Estrogenic (E), 4 patients
Mixed Progestational (M), 8 patients
Progestational (P), 2 patients
Progestational, pregnancy, biopsied (P*), 1 patient
Progestational, presumed due to the occurrence of pregnancy, 2 patients

The incidence of progestational responses, including pregnancies, was equivalent to 76.5%.

Redistribution of these follow-up data among the 15 patients who gave positive responses during treatment cycles and the 14 patients who did not respond yielded these facts. Follow-up endometrial studies were made on 10 of the 15 patients who responded during treatment cycles: all of these 10 patients (100%) yielded progestational endometrial responses after one or two non-treatment cycles had elapsed.

Follow-up endometrial studies were made on 7 of the 14 patients who failed to give progestational responses during treatment cycles: 3 of these 7 patients (45.3%) gave progestational endometrial responses after one or two non-treatment cycles had elapsed.

Alterations in the character of uterine bleeding. The effects of therapy upon the existing irregularities of bleeding or upon cyclic bleeding were investigated in all 31 patients. The immediate effects of each of the 37 cycles of therapy upon the menometrorrhagia of 19 of these patients were:

Bleeding occurred at the calculated time as regards cyclicity of therapy, 12 instances
Oligomenorrhea occurred, 16 instances
Polymenorrhea occurred, 2 instances
No bleeding occurred (cycle longer than 60 days), (one instance was due to intercurrent pregnancy), 7 instances

Therapy had no effects upon the cyclic bleeding of Polymenorrhea occurred, 4 instances
No bleeding occurred immediately following therapy (cycles longer than 75 days), 5 instances

The immediate effects of each of the 15 cycles of therapy upon the oligomenorrhea of 9 of these patients were:

Bleeding occurred at the calculated time as regards cyclicity of therapy, 4 instances
Oligomenorrhea not changed, 2 instances

TABLE 1. COMPOSITE CLINICAL DATA OF THE 31 PATIENTS WHO RECEIVED ONE-TWO CYCLIC THERAPY WITH EQUINE AND CHORIONIC GONADOTROPINS

Case No.	Age	Menstrual History	Endometrium Before Treatment	Nature of Treatment and Endometrial Responses Associated with Bleeding during Therapy	Endometrium After Treatment	Follow-up Data
EG–CG8	35	CyS	E	E(750–3000); E(3375–4200); M(5500–3300)	PP	Pregnancy
EG–CG11	36	CyS	E	M(4500–3300); M(2000–4200)	PP	Pregnancy
B16	28	CyS	E	M(4500–3900); O(3375–4200); O(6000–4200)	M	→CgP
EG–CG3	24	Mm	↓E	E(3500–4200)33	P	OP 15 mo.
EG–CG13	17	Mm	E	O(4125–7200)32; O(2250–3900)37; E(3375–4200)	M	→CgP
EG–CG19	16	Mm	E+	E(3600–3000)35	θ	Menorrhagia. Curettage after 3 mo.
EG–CG22	14	Mm	↓E	E+(4000–3000)40; O(1200–3000)	P	OP 8 mo.
EG–CG42	27	Mm	E	E(2400–3000); E(2000–3000)36	O	OP 6 mo.
B14	20	Mm	E	E(3000–4200); M(3000–3600); E(1875–4200)	M	OP 11 mo.
B10	30	Mm	E	M(5250–2400)57	O	Menorrhagia. Curettage after 1 mo.
B23	15	Mm	E	θ(3600–4200); E(1800–3600)35	Eθ	→CySt
IX	18	Mm	E	E(2400–4200)38; M(2000–3900); E(3600–3000)23	M	OP, 7 mo.→CySt
O15	18	Mm	E	M(4500–3900)	M	OP, 1 year
O17	27	Mm	↓E+	E+θ(2000–3000); O(2000–3000)	—	Menorrhagia
O19	23	Mm	E	E(4000–2700)33; E(3600–3000)31	—	No data
O20	31	Mm	E+	E(4000–3000)35	—	No data
O32	17	Mm	E	E+(5000–4200)34; θ(4000–3000)	E	Menorrhagia
O33	17	Mm	E	E(3200–3000)50; M(3600–3000)45	O	→AhP
O34	29	Mm	E	E(4000–2400)20; E(5000–2100)21; E(3600–3000)20	E	OP 4 mo.
O38	27	Mm	E	M(4000–3000)35; Pθ(2800–3000)	M	OP 4 mo.
O40	17	Mm	E	E(4000–600); O(800–600); M(2000–2100)	M	No data
EG–CG1	18	Ol	E–	O(3000–4800); E–(1125–3300)24; E–(2800–2100)	Eθ	→AhP
EG–CG14	23	Ol	E	Eθ(5000–3300)	θ	No data
B17	28	Ol	E	E(4000–4200); θ(2800–3000)	θ	→AhP
O35	22	Ol	E	M(1600–4200); Eθ(1800–3000)	M	OP 8 mo.
B5	22	Ol	E–	θ(6500–4200); P*θ(4000–3000)	P*	→CySt
EG–CG43	20	Ol	E	M(2800–900)24	—	No data
B13	16	Ol	E	M(3000–2400)55	—	OP 13 mo.
EG+CG44	20	Ol	E	M(3375–4200)47	—	OP 14 mo.
O10	17	Mm	E	θ(5000–4200)75	Eθ	OP 7 mo.
EG+CG15	29	Ol	E–	θ(4875–4200); θ(3750–4200)	θ	→CgP

Menstrual history: CyS, cyclic bleeding, sterility; Mm, menometrorrhagia; Ol, oligomenorrhea. Endometrium at bleeding (or at time when bleeding should have occurred) before, during and after treatment: ↓ curettage prior to therapy; E–, hypoestrogenic endometrium; E, persistent estrogenic endometrium; E+, hyperestrogenic endometrium; M, mixed progestational endometrium; P, normal progestational endometrium; P*, pregnancy demonstrated by chorionic villi in endometrial sample; PP, progestational endometrium, presumed due to occurrence of pregnancy; O, no biopsy; θ, no bleeding occurred after a period of treatment. Numerals in parentheses indicate total cyclic dosage in international units of equine and chorionic gonadotropins respectively. Numerals after parentheses signify length of cycle; absence of numerals indicates length of cycle was within normal range, i.e., 25–30 days. Follow-up data: CgP indicates that the patient was transferred to cyclic therapy with chorionic gonadotropins and progesterone; CySt indicates that the patient was transferred to cyclic sterol therapy administered by injection; AhP indicates that the patient was transferred to cyclic therapy with anhydrohydroxy-progesterone orally administered; OP followed by number of months indicates that no further treatment has been given during the specified interval of months.

3 patients who received 8 cycles of treatment.

The average durations of episodes of bleeding which immediately followed cycles of therapy were 4.7 days for the menometrorrhagia group and 4 days for the oligomenorrhea group.

The follow-up data upon the bleeding histories of the patients of these various groups of patients are summarized:

Of the 3 patients with cyclic bleeding,
- 2 became pregnant; one subsequently aborted,
- 1 continued cyclic bleeding.

Of the 19 patients with menometrorrhagia,
- 8 remained cyclic for times which ranged from 4 to 15 months,
- 4 developed menorrhagia, within 1 to 2 months, which required curettage in two instances and additional therapy in the others,
- 3 gave no follow-up data,
- 4 received additional therapy before sufficient time had elapsed for ultimate evaluation of the one-two cyclic therapy.

Of the 9 patients with oligomenorrhea,
- 3 remained cyclic for times which ranged from 8 to 14 months,
- 3 received additional therapy before sufficient time had elapsed for ultimate evaluation of the one-two cyclic therapy,
- 2 gave no follow-up data,
- 1 was pregnant, but abortion occurred.

A number of these patients was transferred subsequently to other therapeutic schedules, the nature of which is indicated in table 1.

DISCUSSION

These data indicate that one-two cyclic gonadotropic therapy employing equine and chorionic gonadotropins permits the therapeutic salvage of a large group of patients with anovulatory ovarian failure. The unequivocal results obtained with this form of therapy contrast strikingly with the data of the previous communication in regard to the cyclic use of equine gonadotropin alone. Since the patients of both studies were chosen in the same random manner and since the same criteria for investigation and evaluation of results were employed, the only apparent variant was the nature of therapy. This differed, not in the amount or duration of the equine gonadotropic therapy, but only in regard to the added chorionic gonadotropin. It seems reasonable, therefore, to relate the ten-fold increase in responses observed in the present study to a 'synergizing' or an 'augmenting' action of the chorionic gonadotropic portion of the therapy.

The fact that 51.7% of the patients treated gave positive ovarian responses seems to be strong evidence of the specificity of the therapy. These patients are presumed to have experienced ovarian failure due to relative deficiencies of pituitary function, the ovaries of these patients, however, retaining their receptivities to gonadotropic influences. The occurrences of pregnancies during therapy or immediately thereafter in 3 of the 15 patients who were judged to have responded favorably to therapy add weight to the significance of the endometrial evaluations derived from these studies. The fact that 2 of these 15 patients (O_{33} and B_5) had failed previously to respond to the cyclic employment of equine gonadotropin alone permits additional confirmation of the specificity of the one-two system of treatment.

The incidence of relative pituitary failure in over one half of a random group of patients with anovulatory ovarian failure was really higher than anticipated by us. A certain number of the patients studied was expected by chance alone to have ovaries of lowered reactivity, although the ages of the patients studied were against the inclusion of many patients with ovarian refractivity due to the climacteric. This group, however, potentially contained patients with ovarian failures due to diverse causes.

The follow-up data upon the endometrial responses of these patients after discontinuation of therapy were surprising and much more favorable than the earlier impressions gained by one of us (1) from a smaller group of patients. Apparently the results of this form of therapy are less temporary than one would expect from simple substitutional therapy, since all of the patients investigated, who had responded during cycles of therapy, continued to have satisfactory ovarian responses for one to several cycles after therapy was discontinued. Doubtlessly, follow-up studies of these patients by endometrial biopsies for more remote intervals, as regards cessation of therapy, would reveal the occurrence of ovarian failure in some of them. Obviously, the continuation of satisfactory responses after discontinuation of therapy indicates the non-existence of any severe degree of pituitary failure.

These follow-up data, moreover, indicate that delayed positive responses may result from therapy, as manifested by the occurrence after cessation of therapy of progestational endometria in 3 of 7 patients, who had not yielded positive responses during treatment cycles. This delayed effect is not characteristic of simple substitutional therapy and was unexpected from our preconceptions founded on this assumption.

While this therapy apparently resulted in somewhat better regulation of irregular uterine bleeding than did that which employed equine gonadotropins alone, it is not nearly as effective in this regard as cyclic treatment with sex steroids (2). The same lack of correlation between the induction of specific endo-

metrial responses and regulation of bleeding, which was observed in the previous communication, characterized the findings of the present study. Instances were observed in which progestational differentiation of the endometrium was not followed by uterine bleeding. The most likely explanations of these instances may be either the production of hyperluteinized ovaries or an incomplete vascularization of the endometrium. The occurrence of pregnancies, however, is against any general assumptions of the latter nature.

The inadvertent biopsying of an early pregnancy in this group of patients was due to our efforts to evaluate endometrial responses even in those cycles not characterized by bleeding. These efforts led to the findings just cited, that progestational activity of the ovaries may be aroused without subsequent bleeding.

SUMMARY

The responses of 31 women, aged 14 to 36 years, with estrogenic bleeding to one-two cyclic therapy with equine and chorionic gonadotropins were studied. The therapeutic schedule, as a rule, was the following one: equine gonadotropin was given intramuscularly in daily doses of 400 I.U. for 10 days, beginning with the last day of flowing; 10 days of chorionic gonadotropic therapy in daily intramuscular doses of 300 I.U. followed immediately the equine gonadotropic portion of treatment. Of the 29 women whose endometrial data were adequate for evaluation, 15 gave positive responses (progestational endometria) to one or more cycles of therapy (an incidence of 51.7% positively responding patients). After therapy had been discontinued and one to several nontreatment cycles had elapsed, 10 of the 15 positively responding patients investigated continued to show progestational responses and 3 of the 7 negatively responding now showed progestational responses. Pregnancies occurred during or immediately following therapy in 3 of the 15 women responding during treatment cycles. No striking regulatory effects upon the irregular bleeding of 28 of the patients resulted from therapy.

Equine gonadotropin for these studies was supplied by Schering Corporation, Bloomfield, N. J., (Anteron), The Upjohn Company, Kalamazoo, Mich., (Gonadogen) and Cutter Laboratories, Berkeley, Calif. (Gonadin). Chorionic gonadotropin was furnished in liberal quantities by Ayerst, McKenna and Harrison, Montreal, Canada, (A.P.L.).

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Use of Estrogenic Vaginal Suppositories in Vaginitis in Women

[Estrogens in Vaginitis]

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AFTER ALLEN HAD SHOWN that the estrogens bring about thickening (maturity) of the vaginal epithelial layers in immature animals (1), Lewis (2) conceived the idea that "if this change to the adult type of epithelium could be induced in the child's vagina, its gonococcic infection might be overcome." Lewis reported the first experiments with such endocrine therapy in 1933.

Later, having extended his series of cases, Lewis (3, 4, 5) stated that the success of estrogenic therapy is due not only to the histologic changes, with the production of a thicker and partly cornified epithelium, but also to a "striking change in the reaction of the vaginal secretions." The latter, a marked acid reaction, was found to be inimical to the growth of gonococci as well as many other pathogenic bacteria. Of course, both of these changes are closely connected for the change in the epithelium to the adult type is attended by the formation of glycogen in its cells, and glycogen is essential for growth of the Doederlein bacillus and the production of lactic acid.

Following Lewis, many clinicians have employed and studied the use of the estrogens in the treatment of vulvovaginitis in children and infants. In 1937, Benson and Steer (6) reviewed the extensive literature on this project and stated that the majority of reports were favorable. They pointed out, however, that the problem of recurrences was not entirely solved.

When first used, estrogenic treatment for vulvovaginitis in children was administered parenterally, but this form of therapy has been replaced more and more by intravaginal administration by means of estrogenic vaginal suppositories, which have proved to be equally if not more effective than injection of the estrogenic preparations. Lewis, (3, 4, 5) Te Linde and Browner (7), Te Linde (8), Mazer and Schechter (9), Jacoby et al. (10), Labhardt (11) and others have reported very satisfactory results from the intravaginal administration of the estrogens.

Te Linde (7, 8), who has made extensive trials of the estrogens in vulvovaginitis, observed that children who had not responded to parenteral treatment even when given in large doses, were satisfactorily improved by intravaginal therapy. In his experience, there was no case of vaginitis which did not yield to local treatment, and he emphasizes intravaginal administration as the method of choice. Epithelial growth was obtained in the average case within 2 weeks, and vaginal smears were negative for gonococci within less than 3 weeks. A complete course of treatment required on an average about one month. In spite of this short period of treatment there were very few recurrences. In contrast, the former treatment with antiseptics had to be continued for a much longer period of time. (Te Linde at Johns Hopkins found an average of four months necessary, while Lewis of New Haven and Adler of New York reported four and one-half months.) The total dose of estrogen, required to bring about the desired epithelial change with intravaginal administration was found to be approximately one-half of the total dose when administered parenterally. Undesirable side-effects such as breast development, did not occur with intravaginal therapy. This is another proof that the new method is superior to the old.

The attempt has been made to cure gonorrheal vulvovaginitis with acidifying agents alone. Burpee, Robinow and Leslie (12) reported fairly good results with acidic douches or the insertion of vaginal tablets containing mild acids or fermentable carbohydrates. Karnaky (13) has reported on the use of acidifying agents combined with dextrose in the form of tablets containing lactose, dextrose, boric acid and diodoquin; these tablets had to be inserted 2 to 3 times a day for 30 days. Te Linde employed the same

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preparation in a series of 17 cases with the result that remission of the infection was obtained in only 7 of the cases. Apparently, in vulvovaginitis the effect of acidifying agents alone is rapid and of some value, but is only temporary. The addition of a sugar to these agents prolongs the effect somewhat.

The use of estrogenic vaginal suppositories naturally has been extended to patients with senile vaginitis, as in elderly women the vaginal epithelium is thin and poorly developed just as in childhood: "The vaginal mucosa reverts to the thin and ill-developed structure of childhood," say Lewis and Adler (4, 5), "secretions are no longer acid, and the mucosa becomes easily infected." Davis (14) employed estrogenic therapy in senile vaginitis with good results, administering the estrogens both parenterally and intravaginally. He found that intravaginal therapy alone did not work, probably because of the small dose which was used, namely 75 R.U. Lewis and Adler (4, 5) reported better results with the intravaginal administration of larger doses, 1,000 I.U. McGregor (15) found that about 50% of the patients treated with estrogenic vaginal suppositories responded satisfactorily to treatment with epithelial growth and desquamation of the infected tissue followed by healing.

Mishell and Motyloff (16) found an estrogenic ointment "of definite value in the local therapy of senile vulvovaginitis." Finkler (17), with the combined use of vaginal estrogenic suppositories and an oral dose of 2,500 R.U. of estradiol every night for 2 to 4 weeks, found in 10 cases of senile vaginitis that the vaginal epithelium was restored in all, good clinical improvement was shown by 6 patients, moderate clinical improvement occurred in 3, and there was no alteration in the clinical status in 1 case. Foss also has reported satisfactory results from the insertion of vaginal estrogenic suppositories, each containing a large dose of estradiol benzoate (3,600 I.B.U.).

Thus far no attempt has been made, to our knowledge, to evaluate estrogenic therapy in non-specific vaginitis in women during the age of ovarian activity. Presumably the use of estrogenic therapy in vaginal disorders has been restricted to the extremes of life, childhood and old age, because such therapy is supposed to exert a curative effect by supplying the sex hormone that is characteristically lacking at these periods of life. In the majority of cases of simple leukorrhea or non-specific vaginitis adequate local cause cannot be demonstrated and the probability is that a systemic factor is involved, namely, a state of lowered resistance against bacterial infection. It is our belief that such a state of lowered resistance, particularly if it eventuates in a vaginal disorder, is due in no small part to hormonal insufficiency, with lowered blood levels of estrogen. Whether or not the estrogenic deficiency is considerable, if it is a factor at all, the possibility of influencing the disease by supplying estrogen should be considered. We have, therefore, applied estrogenic therapy in cases of non-specific vaginitis developing in women in the so-called period of "sexual maturity," during adult ovarian activity or function.

The patients in whom the estrogenic therapy was applied were private patients, ranging in age from 20 to 47 years, the average being 36.1 years. All patients were in the period of "sexual activity," although 3 were 45 to 47 years old. One patient, a girl, 20 years of age, had a marked genital hypoplasia and infrequent and irregular menstruation. It is of interest that in this case, although the vaginitis was completely relieved, the irregularities in the menstrual flow continued. Another patient had had a hysterectomy a number of years ago; the ovaries, however, were not removed. With but one exception none of the patients had or had had a specific vaginal infection. One patient had gonorrhea many years ago but for the past several years all smears had been free of gonococci before menstruation as well as during the intermenstrual period.

Prior to employing vaginal estrogenic suppositories in the series of cases to be described in this paper, the vaginal pH was determined[1] on every patient reporting for treatment, regardless of the nature of her complaint. It was found that the majority of patients not affected with vaginitis or leukorrhea, had a normal vaginal pH between 4.5 and 6.0. In the case of patients who have had vaginitis or leukorrhea, however, the vaginal pH was weakly acid or definitely alkaline. In 22 cases of vaginitis in women during the period of sexual activity, a pH below 6.0 was found in only one case; in 13, the pH was 6.0; in 4, 7.0; in 4, between 7.5 and 9.0.

The first patients selected for treatment were women whose vaginitis had proved resistant to all other treatment. Results were surprisingly satisfactory in the nature of definite and prompt improvement. With these results, the series of cases was built up fairly rapidly.

It is to be noted that all patients, being private cases, were engaged in professional or domestic duties which were continued throughout their treatment. The pressure of these duties and the fact that strict observation of frequent appointments could not be insisted upon with private patients offered some difficulty in that the patients were not seen as frequently as desired. Consequently, it was ad-

[1] Vaginal pH was determined by use of Nitrazine or Hydrion paper.

vantageous to employ treatment which need not be administered by injection or require frequent personal supervision by the physician. The preparation chosen was a vaginal suppository[2] containing 1,000 I.U. of purified estrogenic substance in glucose with sodium phosphate. Each patient was instructed to insert one suppository into the vagina at bedtime. Local treatment of any nature, particularly douches, was proscribed. Accumulation of secretions in the introitus, if present, could be removed by swabbing. A few patients were instructed to insert the first suppository during the day and to note the time required for its disintegration. This was reported to be within 2½ to 8 hours. In several cases the suppositories were used only every second night with satisfactory results. In the main, however, the best and most prompt results were obtained from the daily use of the suppositories. After subsidence of symptoms, treatment was continued 1 to 2 weeks longer; the total course of treatment required 3 to 4 weeks.

The change effected by therapy in the vaginal pH was definite and prompt. This was most striking in those cases in which the pH of the vagina was decidedly alkaline prior to treatment. A shift to an acid reaction occurred following even the first suppository. The alteration in the vaginal pH was from 8.5 or 7.5 to 6.0 or 5.0. Six patients whose vaginal pH was acid before treatment (6.0 to 5.0), showed a shift in the pH to a more marked acidity. Three patients whose vaginal pH was 6.0 to 5.0 before treatment maintained this without change throughout the treatment. In all patients the shift in the pH to the acid side, once established, was maintained even if treatment was withheld for a few days.

Simultaneously with the change in vaginal reaction definite improvement of the vaginitis occurred. The discharge abated, injection and congestion of the vaginal mucosa remitted, and there was cessation of itching, burning, and other subjective symptoms. As in the case of the vaginal pH, the favorable change was most marked in the cases in which the vaginal reaction was definitely alkaline prior to the institution of treatment. The improvement in the vaginitis was more gradual in those cases in which the vaginal reaction was not definitely alkaline prior to treatment. In all cases satisfactory improvement was obtained within one week.

Out of a total of 22 patients treated, 13 were completely freed of all symptoms within 2 to 3 weeks. Four improved within one week, particularly as to vaginal discharge, and failed to report for further observation thereafter. One patient, a very nervous woman, stopped treatment after the insertion of the first suppository complaining that the itching was aggravated. This complaint was not encountered in any of the other cases and no other reactions or by-effects were noted. The remaining 4 patients of the series showed decided improvement but there was a persistence of the vaginal discharge. In all 4 of these cases there were cervical erosions which were cauterized, and prompt cessation of the discharge followed.

Of the 13 patients who were completely freed of all symptoms both subjective and objective, 2 had a recurrence of the vaginitis several months later. In both cases, the vaginitis disappeared after a 12 day course of treatment, using one suppository daily. Both patients stated that the recurrence developed following personal difficulties and emotional stress.

The following case histories are presented for the purpose of demonstrating the effectiveness of the therapy. All of the cases included in this study ran a similar course.

Case 1, Mrs. E.S., age 33 years. Vaginitis and leukorrhea for 14 years. All treatments advocated for these disorders had been employed without success. After the use of 6 vaginal suppositories, one each night, the vaginal discharge ceased, the vaginitis cleared, and there were no further complaints. Three to 4 months later after an emotional crisis, the discharge and vaginitis recurred. After the insertion of one vaginal suppository daily for 12 days, the disorders again abated. Six months following conclusion of the second course of treatment, there were no signs of disease and no complaints.

Case 2, Mrs. H., age 37 years. Profuse yellowish-white vaginal discharge. After the use of 6 vaginal suppositories, one inserted every second night, the discharge ceased and there were no signs of vaginitis. The vaginal pH shifted from 9.0 to 6.0. The use of the suppositories, every night, was continued for 3 weeks. Six months following conclusion of treatment, symptoms had not recurred, examination showed no signs of disease and there were no complaints. There was a history of gonorrheal infection many years ago, but the exact number of years could not be determined. Vaginal smears for gonococci had been negative for the past several years.

Case 3, Mrs. R.K., age 26 years. Vaginitis. Complete remission of symptoms after the use of one suppository daily for 6 days. Two months later there was a mild recurrence which yielded to another 6-day course of treatment. Four months following the second course of treatment, the patient had had no further trouble.

Case 4, Mrs. Ha., age 38 years. Vaginitis with profuse discharge. The vaginal discharge stopped after insertion of the first suppository. Eleven more suppositories were used, one each night. The vaginal pH shifted from 8.5 to 5.0 and there were no signs of vaginitis at the conclusion of treatment. Four months later no objective signs were

[2] Kolpon Inserts manufactured by Roche-Organon, Nutley, New Jersey.

discovered upon examination and there were no complaints.

Case 5, Miss A.R., age 44 years. Leukorrhea and psychoneurosis. After the use of 6 suppositories the discharge ceased. Treatment was discontinued against medical advice. Four weeks later there was a relapse which was overcome by the use of a second series of 6 suppositories. Patient again discontinued treatment of her own accord and 2 weeks later suffered another relapse. During the following year this happened again and again; due to the mental instability, the patient stopped the treatment as soon as the discharge ceased. It reappeared with each period of worry and promptly disappeared after the use of a few suppositories, proving the latter's efficacy in leukorrhea of constitutional origin.

Case 6, Miss Mi., age 20 years. Genital hypoplasia and vaginitis. Following the use of one suppository daily for 12 days, the vaginitis was cured. The vaginal PH before and after treatment was 6.0. There was no systemic effect from the intravaginal application of the estrogens. No relapse.

Case 7, Miss Na., age 23 years. Leukorrhea and anemia. After the use of one vaginal suppository daily for 6 days, the discharge ceased. No relapse.

DISCUSSION

In the search for the most suitable treatment for any condition, the physician's first aim should be to find the cause of the complaint. It always will be true that to remove the cause is to remove the complaint. In cases where a vaginal discharge is the principal or, perhaps, the only complaint, the causes may be found in:

1) Local abnormalities, such as lacerations of the perineum, descensus of the uterus or of the vagina, erosions, or other anatomical changes.

2) Constitutional and endocrine disturbances manifested by degreees of weakness, physical and mental.

In the case of patients with disorders of the type in the first category, the abnormalities should be corrected by appropriate operative or conservative measures. In cases coming within the second category, however, general treatment in order to build up the system physically and/or mentally is the indicated measure. For this purpose the use of estrogenic suppositories has afforded excellent results. Not only was the PH of the vagina and the local pathology corrected but in almost all cases a very satisfactory improvement in the general condition of the patients was observed.

In the evaluation of any such treatment for nonspecific vaginitis or leukorrhea, it must be borne in mind that these disorders are practically always related to the physical make-up and to the nervous or emotional status of the patient. Local vaginal disorders are, therefore, decidedly affected by environmental changes. Attention has been directed to cases in which recurrence of the distressing symptoms were associated with a period of worry and excitement. On the other hand, improved or more favorable environmental conditions may be attended by an improvement in the genital disorder. For these reasons, general supervision of the patient to assure adequate rest, sleep, nourishment, and controlled exercise, is important. Overexertion and excitement are strictly forbidden. Anti-anemia therapy is frequently necessary. These measures are the general adjuvant background for vaginal estrogenic therapy, and they were employed, but without definite result, prior to the use of estrogenic suppositories, in all cases in our series.

In cases in which the reaction of the vagina was decidedly alkaline, improvement after the institution of vaginal estrogenic therapy was so prompt and definite that undoubtedly the vaginal therapy was the decisive factor in eradicating the disorder. In cases in which the vaginal PH was 6.0 or less, the effect of the vaginal estrogenic therapy on the genital disorder, although less prompt, was without doubt an important factor in the recovery. The general therapeutic measures alone, previously proven to be ineffective, could not have been responsible for the definite improvement which was observed.

Vaginal estrogenic therapy has the advantage of providing psychotherapeutic as well as physiologic and chemical effect. The patient experiences a welcome feeling of relief upon cessation of the vaginal discharge which has bothered her over a long period of time, and that constitutes the psychotherapeutic effect mentioned above. It is difficult to say whether the favorable local effect is a 'sedative' to the mentally and nervously unstable woman, or whether the local improvement is a consequence of her faith in the efficacy of an unusual kind of treatment. The fact remains that the results were highly satisfactory. Whether the small quantity of estrogen, absorbed into the system during the intravaginal application, contributed to the mental improvement we do not know.

SUMMARY

The use of vaginal estrogenic suppositories in the treatment of vulvovaginitis in children and in women in or beyond the climacteric afforded satisfactory results.

The success of the therapy is believed to have been due to the induction of changes less favorable to the growth of pathogenic bacteria and assuring more resistance to infection, through the production of a

healthier vaginal mucosa with hyperplasia and cornification of the epithelium and an acid cervical and vaginal secretion.

Non-specific vaginitis and leukorrhea in women during the period of sexual maturity is frequently accompanied by diminished acidity of the vaginal secretions, hence it is reasoned there may be present a mild estrogenic deficiency.

In our investigation, the presence of such deficiencies were accepted as a fact. They were obviously corrected by the use of vaginal suppositories containing 1,000 I.U. of purified estrogen in glucose with sodium phosphate, in our series of 22 cases of non-specific vaginitis in women in the period of sexual maturity. Thirteen patients were cured within 2 to 3 weeks; 8 patients were markedly improved (4 of this group discontinued treatment prematurely and the other 4 had to have cervical cauterizations to completely eradicate vaginal discharge); one patient discontinued treatment immediately after its institution.

Vaginal estrogenic therapy, applied by daily insertion of a vaginal suppository containing 1,000 I.U. of purified estrogen in glucose with sodium phosphate, proved adequate and satisfactory treatment for uncomplicated cases of non-specific vaginitis.

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Suitable Dosage of Stilbestrol for Suppression of Lactation[1]

[Suppression of Lactation]

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INHIBITION OF LACTATION during the postpartum period, with freedom from severe breast pains, has been successfully effected by various means, including camphor-in-oil, estrogenic hormone and male hormone. The value of the oral administration of stilbestrol to accomplish the same results was then investigated.

Lindemann (1) and Adrian (2) found that large doses of estrogenic hormone would bring about diminished secretion of milk in the postpartum woman. They gave 60,000 to 200,000 I.U. of follicular hormone within 2 to 3 days. Foss and Phillips (3) gave estradiol orally in the dosage of 100,000 I.U. a day for 1 to 4 days with apparently successful results. However, there were no observations made on the patients after they left the hospital.

Inhibition of lactation by stilbestrol has been described by Winterton and MacGregor (4) by Wenner and Joel (5) and by Bishop et al. (6). Winterton and MacGregor gave 5 mg. per day to 7 patients and were successful in three. Wenner and Joel obtained complete absence of lactation and breast pain in 6 cases with administration of 5 to 10 mg. in one dose on the first day postpartum, and in 13 of 16 cases, in which lactation had already begun, complete relief was obtained after treatment. In two of their cases lactation was arrested with 5 mg. but after 1 to 2 days the breasts became distended and painful. They mentioned the fact that none of their patients presented any of the toxic symptoms which sometimes accompany stilbestrol medication. Bishop obtained uniformly successful results in inhibiting lactation in 4 women using 5 mg. of stilbestrol for 4 to 6 doses. Again, none of the authors who used stilbestrol mention observation of their cases after discharge from the hospital.

MATERIAL

The material for this study comprised 120 private patients of members of the staff of the Bronx Hospital. Ninety-one patients received stilbestrol[2] before the onset of lactation. This constitutes *group 1*. The patients in *group 1* were further subdivided into) group *1a*, *1b*, and *1c* depending upon the dosage and number of days the drug was administered. Twenty-nine women included in *group 2* were given the drug after lactation had become established. No other therapeutic means, such as sedatives, tight binders, cathartics or limitation of fluids were employed. Observations of the inhibition of lactation and prevention or relief of breast engorgement were made daily in the hospital. During the 6th week postpartum the various private physicians submitted a follow-up report on each of the 120 patients.

The indications for inhibition or cessation of lactation in this series are given in table 1.

RESULTS

Group 1a (table 2) consisted of 3 women who were the first patients to receive stilbestrol for the suppression of lactation. Since the dosage and period of administration were not known at the time, the patients arbitrarily were given 10 mg. the first day and 5 mg. every day thereafter for 3 days, a total of 25 mg. In each instance the breasts were soft and nonlactating while the drug was taken but began to secrete milk and became painful within 4 to 5 days after cessation of the drug. The only effect stilbestrol had in this group was to postpone the onset of lacta-

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[1] The material in this paper was presented at a meeting of the Bronx Obstetrical and Gynecological Society, Nov. 25, 1940.

[2] Stilbestrol, under the trade name, Estrobene, was supplied through the courtesy of Ayerst, McKenna & Harrison, Ltd., Montreal, Canada.

tion. The following case is typical of this group.

K.V., a para 0, gravida 1, had a spontaneous delivery Jan. 21, 1940. She did not wish to nurse her baby. She was given 10 mg. of stilbestrol January 22, and 5 mg. a day for the next 3 days. The last dose was received on Jan. 25. The breasts remained soft until Jan. 30, at which time they became painful and firm. The pain remained for 48 hours and was moderate in intensity.

TABLE 1. INDICATIONS FOR INHIBITION OR CESSATION OF LACTATION. *Group 1*, 91 patients, received therapy before the onset of lactation, *subgroups a, b,* and *c* indicating variations in dosage. *Group 2*, 29 patients, received therapy after the onset of lactation.

Indications	Groups			
	1a	1b	1c	2
Mother does not wish to nurse	2	8	20	8
Physician prefers bottle	0	7	15	2
Insufficient milk with 1st baby	0	0	5	1
Fetal death	1	4	6	3
Neurosis	0	0	2	0
Fissured, flat or inverted nipples	0	1	3	3
Mother must return to work	0	0	4	1
Previous breast abscess	0	0	2	1
G. U. Infection	0	0	2	0
Pulmonary T. B.	0	0	1	0
Fetus isolated for impetigo	0	0	3	0
Cardiac	0	2	0	0
Spontaneous pneumothorax	0	1	0	0
Pneumonia	0	1	0	1
Mastitis	0	0	0	2
Postpartum infection	0	0	0	2
Insufficient breast milk	0	0	0	4
Excessive loss in baby's weight	0	0	1	1
Total	3	24	64	29

Group 1b (table 2) was made up of 24 women who were given 10 mg. of stilbestrol the first day of treatment, followed by 5 mg. a day for 8 to 9 days, a total of 50 to 55 mg. The initial dose was given the first or second day postpartum. Since the length of the hospital stay on the maternity service is 10 days, more than 10 daily doses could not be given. The number of daily doses was increased in this group in an attempt to prevent the occurrence of lactation after the drug was discontinued (as noted in *group 1a*). Of the 24 patients in this group, 3 experienced painful lactating breasts and 21 had no pain while in the hospital; there was complete success in 87.5% of the cases treated during the 10 days postpartum. Of the unsuccessful cases, one had severe pain for 2 days, one had moderate pain for one day and one had slight pain for 6 hours. Of the 21 successful cases (those that experienced no pain) 11 presented other breast findings; one had firm breasts, without pain, on the 17th day; 4 had galactorrhea which began on the 5th to the 10th day postpartum and lasted from 1 to 14 days; 2 had firm breasts and galactorrhea on the 10th and 12th days postpartum for 1 and 2 days respectively; 4 of the patients who had no breast symptoms in the hospital, subsequently developed painful lactating breasts on the 14th day postpartum. The course of the latter group was similar to that of an untreated case, the patients complaining of moderate to severe pain for 2 to 3 days. The following case is illustrative of this group.

A.W., 28 years old, para 2, gravida 3, had a spontaneous pneumothorax while delivering her baby on Jan. 22, 1940. She was given 10 mg. of stilbestrol on Jan. 23, the first day postpartum and then 5 mg. a day for 8 days. The patient was observed after transfer to the medical service until the 25th day postpartum. Her breasts remained soft and free from pain until 5 days after the drug was discontinued. Then the breasts became painful, hard and began to lactate.

Group 1c (table 2) consisted of 64 patients who were given 10 mg. of stilbestrol the first day, then 5 mg. daily for 8 to 9 days, followed by 1 mg. a day for 10 more days at home, each receiving a total of 60 to 65 mg. The addition of the 10 one-mg. doses was instituted to avoid if possible the unpleasant onset of lactation and breast pain after being discharged from the hospital as had happened in the patients in *group 1b*. Of the 64 women who received the drug in this dosage, only 5 complained of breast pain. This was 92.1% success for the group while in the hospital.

TABLE 2. RELATION OF DOSAGE OF STILBESTROL TO PREVENTION OF BREAST PAIN DURING THE 10-DAY POSTPARTUM HOSPITALIZATION PERIOD AND AT HOME

Dosage Stilbestrol	No. Cases	In hospital, 10 days postpartum			After discharge from hospital
		Painful Breasts			
		Yes	No	% Success	Painful Breasts
Group 1a 1st day 10 mg. followed by 5 mg. a day for 3 more doses, 25 mg. total.	3	3	0	0	0
Group 1b 1st day 10 mg. followed by 5 mg. a day for 8 or 9 doses, total of 50 to 55 mg.	24	3	21	87.5	4
Group 1c 1st day 10 mg. followed by 5 mg. a day for 8 or 9 doses. 1 mg. a day at home from the 11th to 20th day postpartum. Total 60 to 65 mg.	64	5	59	92.1	1
Total	91	11	80		5

Of the 5 unsuccessful cases, 3 had slight pain for 12 hours and 2 had moderate pain for 24 hours. One patient had breast pain after being discharged from the hospital; pain occurred on the 11th day, was moderate in intensity and remained for one day. Thirteen of the successful cases presented other breast findings; 9 had firm breasts without pain, with the onset from the 2nd to the 7th day and lasting for 6 to 30 hours; 2 had galactorrhea beginning on the 3rd and 9th day postpartum and lasting 2 and 7 days respectively; 2 had galactorrhea and firm breasts but no pain, with onset on the 10th and 12th day and for 1 and 2 days' duration respectively.

Table 3 indicates the results in *group 2* when the drug was administered after lactation had set in. Of this group, 76% had relief of breast pain within 24 hours. Three of these women were given the drug 8 hours before weaning and they did not experience any breast discomfort at all. The dosage in this group was 10 mg. the first day of therapy followed by 5 mg. daily until the patient was discharged from the hospital, a total dosage of 20 to 55 mg. In every case the stilbestrol was continued after the symptoms disappeared. Of the 24 patients, 10 received 10 one-mg. daily doses at home. Two of these 10 patients experienced mild, painful breasts at home for 6 and 24 hours on the 20th and 19th days respectively. Of the remaining 14 patients who did not receive the drug at home, one woman experienced moderate breast pain on the 13th day postpartum. The continuation of stilbestrol therapy therefore did not reduce the incidence of breast pain at home in this group. One patient in this group had galactorrhea from the 3rd to the 10th day postpartum. Included in this group were 2 cases of non-suppurative mastitis with hyperpyrexia which began 15 and 67 days postpartum respectively. They responded to stilbestrol therapy within 48 hours. To illustrate the efficacy of the therapy in mastitis the following case is mentioned.

TABLE 3. RELIEF OF BREAST PAIN BY STILBESTROL AFTER ONSET OF LACTATION. 76% reported relief within 24 hours following initiation of treatment

Number of hours elapsing before complete relief of breast pain	Number of cases
0–12	10
13–24	12
25–36	0
37–48	5
49–60	0
61–72	2
Total	29

A.C., complained of a painful right breast for one week when seen for the first time on the 74th day postpartum. The temperature was 105°F., and the right breast contained a tender reddened indurated area about 6 cm. in diameter just medial to the nipple. She was given 10 mg. of stilbestrol the first day followed by 5 mg. a day for 8 days. Within 24 hours the pain was almost completely gone and the patient's temperature had dropped to 101°F. The temperature remained normal after the first day of therapy and the breast became soft and painless.

Vaginal bleeding occurred in 15 of the 120 patients within the period of the 10th to the 45th day postpartum. However, only 7 had excessive bleeding and the remaining 8 had as much as or less than that in a normal menstrual period. Of the 7 patients with excessive bleeding, one occurred in *group 1b*, 3 in *group 1c* and 3 in *group 2*. The bleeding although excessive was at no time alarming, and was probably due to the withdrawal of the estrogenic substance. Bleeding occurs also in the non-pregnant patient after the cessation of administration of large doses of estrogenic hormone.

There were 5 patients who presented mild toxic symptoms following the administration of stilbestrol. One patient vomited within 5 hours following the ingestion of 10 mg. of the drug. Two women complained of mild headache; 2 other women experienced severe abdominal cramps in the region of the uterus (probably uterine contractions). With the exception of the patient who vomited it was unnecessary to discontinue the use of the stilbestrol and the symptoms disappeared.

DISCUSSION

Since stilbestrol is a powerful estrogenic substance, it can be assumed that its action in suppressing lactation is similar to that of any of the natural estrogens. Estrogenic substances have the ability to depress the various hormones secreted by the anterior pituitary gland. In the menopause associated with hyperthyroidism, Goldman et al. (7) has demonstrated that estradiol benzoate causes a reduction of the B.M.R. probably by depressing the excessive production of thyrotropic hormone produced by the anterior pituitary gland. The large amounts of gonadotropic hormone excreted by the castrate woman can be made to disappear by the administration of estrogenic hormone. The lactogenic hormone is probably depressed similarly, by the ingestion of stilbestrol or the injection of natural estrogens. In the postpartum patient the prolactin production must be depressed with stilbestrol for at least 20 days to prevent re-formation of the lactogenic hormone after cessation of therapy. If stilbestrol is discontinued very early in the puerperium, prolactin is likely to return and produce lactation with engorgement and painful breasts.

SUMMARY

Stilbestrol was administered orally to 120 postpartum patients to inhibit lactation and relieve the pain of engorged breasts.

Stilbestrol in suitable dosage starting before the onset of lactation prevented the occurrence of painful breasts in 59 of 64 patients, or 92.1%. It was necessary to continue the administration of the drug longer than the period of 10 days postpartum to obtain successful results.

The administration of stilbestrol after lactation had already begun resulted in complete relief of painful breasts within 24 hours in 22 of 29 patients, or 76%.

The toxic effects of stilbestrol in the postpartum patient in the dosages given are practically nil.

Seven or 5.9% of the 120 patients had excessive postpartum uterine bleeding.

CONCLUSION

Stilbestrol, orally, is an effective drug in suppressing lactation, and preventing engorged and painful breasts in the postpartum patient. Its ease of administration and inexpensiveness make it the choice over other equally effective forms of treatment.

1749 Grand Concourse

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Urinary Excretion of 17-Ketosteroids in Ovarian Failure

I. In Hirsutism and Virilizing Syndromes[1]

[[17-Ketosteroids]]

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During the past three years various members of our group have been engaged in a study of the gynecic roles of androgens. A number of reports of these studies have dealt with the urinary excretion of 17-ketosteroids under diverse conditions (1–6). The present series of communications considers the levels of excretion of these compounds in various grades of ovarian failure. I. In hirsutism and in virilizing syndromes; II. In functional menometrorrhagia; III. In amenorrhea and IV. During the climacteric.

These studies are reported for the purpose of discussing and defining, if possible, the reciprocities in function which may exist between estrogenic function of the ovaries and androgenic function of the adrenal, that is, the hormonal relations of the gonado-pituitary and adreno-pituitary axes. Previous studies by our group have suggested that secondary or late ovarian failure (estrogenic type) may be associated with increase in androgenic function of the adrenal (2, 6).

Review of Some Previously Reported Studies

Source of the urinary androgens of women. Although there is a mass of data from studies on experimental animals which points to the ovaries as the source of androgens in the female, most of the clinical observations suggest that the adrenals are the chief site of formation in men and women. This clinical evidence may be summarized as follows. Normal men and women excrete qualitatively identical androgens in approximately similar amounts. The level of excretion is apparently higher in some eunuchoids than in normal males; in true eunuchs the amounts excreted are low, but not outside of normal limits. Oöphorectomized women, immediately following operation, may excrete smaller quantities than the average normal but values overlap normal range. The excretionary values in Addison's disease have been found very low, while excessive amounts of androgens are excreted by patients with adrenal cortical tumors.

The fact that normal women excrete androgens in amounts essentially the same as normal men was established first by Womack and Koch (7) and later confirmed by various workers using bioassay methods (8–18) and colorimetric methods (19–24).

Callow and his group (14, 25) reported that while eunuchoids may excrete androgens and 17-ketosteroids in amounts which are lower than average values for normal males, some individuals excrete 17-ketosteroids in greater amounts than normal. Engel, Thorn and Lewis (26) found low values for 17-ketosteroids in these patients while Koch (27), Kenyon and co-workers (18, 28), Dorfman and Hamilton (29), and McCullagh, Rumsey and Cuyler (30), employing bioassay methods, found the androgenic output of these patients lower than normal.

In true eunuchs the excretion of androgens has been reported to be lower than normal (14, 18, 30–33). An occasional high androgenic titer was observed (14, 25). The 17-ketosteroid values in these patients were found to be within normal range by Chou and Wu (19), and Callow, Callow and Emmens (14), the latter workers reporting that an occasional individual excreted amounts in excess of normal.

Our group has reported that the excretion of 17-keto-

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steroids is not altered significantly from the normal in recently oöphorectomized women (2). Callow, Callow and Emmens have made similar observations (14).

Others (14, 24, 33) have reported that the excretion of androgens and 17-ketosteroids was depressed markedly in patients with Addison's disease. Callow, Callow and Emmens (14) found decreased urinary titers of androgens and 17-ketosteroids in a man with hypopituitarism. McCullagh and Lilga (33) described low androgenic values in adult males with apparent hypopituitarism.

Numerous investigators have found excessive amounts of androgens and 17-ketosteroids in the urine of women with adrenal virilizing syndromes (23, 34–42).

In regard to the possible site of steroid production, a hypothesis has been advanced (43) that the adrenal and gonadal steroids are all derived from a mother substance produced in the pituitary, believed to be pregnandiene Δ4-8-diol-17-21-trione 3-11-20. The specific steroids are thought to be produced by the reducing action of ascorbic acid upon this common precursor.

Nature of urinary androgens. The androgens which occur in the urine differ chemically from those isolated from the endocrine glands. Testosterone has been recovered only from bull testes (44). Adrenosterone (45) and other closely related steroids (46–48) have been isolated from beef adrenal cortex. Androstanedione has been extracted from boar testes (49). The chemical identity of the androgen in human testes has not been established. The urine of normal men has been shown to contain androsterone (50, 51), dehydroisoandrosterone (52, 53) and the inactive steroid, etiocholanol-3(α)-17-one (54). The identification of these three 17-ketosteroids in the urine of normal women demonstrates that no differences between the two sexes exists in this regard (12, 21, 55). The chemical natures and distributions of urinary 17-ketosteroids in men and women are summarized in table 1.

The urinary levels of 17-ketosteroids tend to be slightly lower in women than in men; the ranges overlap, however, androsterone and dehydroisoandrosterone are excreted by both sexes in a ratio of approximately 1 to 1 (12, 21, 52, 53, 55). The excretion of dehydroisoandrosterone is reported to be greatly increased in cases of adrenal cortical tumors (38, 39) and in adrenal cortical hyperplasia (40). In one case of adrenal cortical tumor Callow (38) traced 70% of the androgenic activity of the urine to dehydroisoandrosterone. This compound is reported to be excreted in amounts greater than normal in some castrated men and women (14). Etiocholanol-3(α)-17-one is found in small amounts in the urine of normal and castrated men and women (see table 1 for references).

Butler and Marrian (62, 63) isolated isoandrosterone from the urine of a woman with adrenal cortical hyperplasia. For some time it was thought that this compound was only excreted in adrenal cortical hyperplasia of females. Recently, however, Hirschmann (65) recovered this compound from the urine of a virile woman who was said not to have had any adrenal cortical pathology. Pearlman (64) recovered it from the urines of normal females and from those of patients of both sexes with carcinoma.

Burrows and his associates (66,67) have isolated and identified Δ3:5 androstadiene-17-one from the urine of a man who had an adrenal cortical tumor. As yet, this is the only one of these excretory products which is identified solely with the male and with adrenal cortical pathology.

The androgenic activities of the urinary non-phenolic 17-ketosteroids may be summarized as follows.

Androsterone	10	I.U./mg.
Dehydroisoandrosterone	3.3–5.0	I.U./mg.
Etiocholanol-3(α)-17-one	0	I.U./mg.
Δ3:5 Androstadiene-17-one	2.5–2.8	I.U./mg.
Isoandrosterone	1.6–2.0	I.U./mg.

Callow (56) has maintained that only 2 of the three 17-ketosteroids found in normal urines, that is, androsterone and etiocholanol-3(α)-17-one, can be regarded as derived from the metabolism of testosterone. Dehydroisoandrosterone must be regarded, even in the male, as being of primary adrenal origin.

17-Ketosteroids and androgens. Their relationships and measurement. By definition androgens must be assayed by biologic methods which test and measure their specific hormonologic effects. When urine ex-

TABLE 1. URINARY 17-KETOSTEROIDS IN NORMAL AND ABNORMAL CONDITIONS IN MEN AND WOMEN, AS REPORTED IN THE LITERATURE

	Androsterone	Dehydroisoandrosterone	Etiocholanol-3(α)-17-one	Isoandrosterone	Δ3:5 Androstadiene-17-one
Normal ♂	(26, 50, 51, 56, 57)	(26, 52, 53, 56)	(13, 26, 56)		
Castrated ♂	(25, 53, 58)	(25, 53, 58)	(25)		
Normal ♀	(21, 57)	(21, 57)	(13, 21, 57)	(64)	
Oöphorectomized ♀	(53, 59, 60)	(14, 59, 60)	(59, 60)		
Adrenal cortical hyperplasia		(40)	(62, 63)	(62, 63)	
Adrenal cortical tumor in ♀		(40)			
Adrenal cortical tumor in ♂					(66, 67)
Virilism				(65)	
♂ and ♀ with cancer				(64)	
Pregnancy	(61)				

tracts are quantitated for androgens, the active 17-ketosteroids are measured.

The term 17-ketosteroid was applied by Callow, Callow and Emmens (20) to those steroids with a ketone group at the 17th carbon atom and a free methylene group which, in the presence of an alkali, unites with meta-dinitrobenzene to produce a characteristic color reaction. This reaction, first described by Zimmermann (68, 69) is not specific for androsterone or any individual 17-ketosteroid. It has been shown (70, 71, 72), however, that the 3-, 6-, and 20-ketosteroids develop absorption bands which are entirely different from those developed by the 17-ketosteroids.

The 17-ketosteroids, which form the urinary products of androgenic metabolism, are contained in the neutral fraction (non-phenolic) fraction of hydrolyzed urine. Estrone, which is a 17-ketosteroid, is eliminated together with the acidic and phenolic fractions by washing the extracts with an alkali. The non-phenolic 17-ketosteroids which are present in the neutral extracts have been divided into 'alpha' and 'beta' ketosteroids (73, 74). These terms refer to the spatial position of the 3-hydroxyl group. The 'beta ketosteroids,' which include dehydroisoandrosterone and isoandrosterone, are precipitated by digitonin. The 'alpha ketosteroids,' which include androsterone, the biologically inactive etiocholanol-3(*a*)-17-one, are not precipitated with digitonin. Under normal conditions in men and women the 'alpha ketosteroids' account for most of the androgens which are bioassayed and the 'beta ketosteroids' comprise the steroids of low androgenicity.

Colorimetric methods, which employ the Zimmermann reaction, may be used to measure the total 17-ketosteroid content of the neutral extract of hydrolyzed urine or to measure the ketosteroid content of the fractions of this extract obtained by precipitation with digitonin. Under either condition, inactive and active 17-ketosteroids are measured.

Callow, Callow and Emmens (20) were of the opinion that, since androgens under normal and most abnormal conditions contribute the greater part of the chromogenic property of urinary extracts, there existed a significant correlation between the results obtained from bioassays and from colorimetric determinations. Friedgood and Whidden (75) and others have concurred in this opinion. Holtorff and Koch (24) examined 41 pure compounds and found that among them the 17-ketosteroids produced the most pronounced absorption in the green. They agreed with Callow, Callow and Emmens (20) that the meta-dinitrobenzene reaction could be used to measure 17-ketosteroids but cautioned that other ketones might add to the color intensity. They were also in agreement with the London group when they concluded that "the absorption spectra of urine extracts are similar to those of 17-ketosteroids, which is good evidence that these substances are producing the color in urine extracts."

Friedgood and Whidden (75) expressed the opinion that, with our present knowledge of 17-ketosteroids, results obtained on daily urinary excretion by colorimetric methods could be expressed in terms of milligram equivalents of crystalline androsterone. This is practically the same as the 'milligram sterone' unit of Callow, Callow and Emmens (20), which refers to the amount of urinary ketonic chromogens in terms of milligram equivalents of crystalline androsterone. A number of workers has adopted the point of view of Friedgood and Whidden, while others, like our group, express their results in terms of international units of androsterone.

Levels of urinary excretion of androgens and 17-ketosteroids in normal females. (*a*) *During infancy, childhood and adolescence.* Despite the fact that extracts of fetal adrenals, which characteristically show marked hyperplasia of the intermediate or x-zone, have shown no androgenic activity (76), the urinary excretion of androgens begins during the early years of life. Androgens have been recovered from the urine of girls as young as 3 years of age (77). During childhood and preadolescence, no specific sexual differences occur in the amounts of urinary androgens. There is a gradual increase in androgenic excretion from early childhood up until adolescence and the attainment of sexual maturity (23, 78). A definite relationship has been established between the 17-ketosteroid excretion, the degree of somatic and sexual development and the chronologic age. In general, it may be said that up until the attainment of sexual maturity the average daily 17-ketosteroid excretion, expressed in I.U. of androsterone, corresponds numerically to the chronologic age.

TABLE 2. URINARY EXCRETORY VALUES FOR ANDROGENS AND 17-KETOSTEROIDS DURING CHILDHOOD AND ADOLESCENCE

Investigators	Age, yr.	Androsterone	
		mg./ 24 hr.	I.U./24 hr.
Bioassay of androgens			
Koch (16)	6–10		0.7– 2.0
Dingemanse, et al. (10)	5–14		5.0– 12.0
Dorfman, et al. (79)	8–12		2.0– 7.0
Colorimetric assay of 17-ketosteroids			
Oesting, Webster (77)	3–14		2.0– 23.0
Chou, Wu (19)	<10	0.2– 1.1	2.0– 11.0
	10–15	0.6– 2.9	6.0– 29.0
Nathanson, et al. (78)	7–12	0.4– 1.8	4.5– 18.0
Talbot, et al. (23)	4– 7	0.8– 2.6	8.0– 26.0
	7–12	1.8– 5.0	18.0– 50.0
	12–15	5.0–11.3	50.0–113.0

Table 2 summarizes the urinary excretory values for androgens and 17-ketosteroids during childhood and adolescence, as reported by various observers.

(*b*) *During the reproductive period.* Table 3 summarizes some of the data reported by various investigators upon the urinary excretory values of

androgens and 17-ketosteroids by women during the reproductive period.

The titration data secured by our group have been consistently lower than those of other workers, both for normal women and for many of the diverse groups of patients investigated. In all 5,700 determinations have been made on 219 women.

In the data of our group for normal women, this general tendency is well illustrated. Several explanations exist. The method of extraction used by us is thought to be less effective in removing non-ketosteroid chromogens from the urine than some used by other workers. The selection of the group of 13 normal women, upon which these data were based, was very critical. The criteria for normalcy included: excellent level of general health; satisfactory physical examination; the absence of any signs or symptoms of endocrine disease; an age range of 20 to 38 years; cyclic and quantitatively normal menses; the normal urinary excretion of the pregnandiol complex; and, in most instances, the association of uterine bleeding with well proliferated progestational endometria.

Despite the fact that no definite cyclicity in the urinary excretion of 17-ketosteroids during various phases of the menstrual cycle has been established, some variations between the data of various observers may be accounted for upon such a theoretical basis. Some workers have reported the apparent occurrence of higher titers premenstrually (10, 35, 82); others have thought greater amounts were excreted postmenstrually (12, 34). Our group has found the average values for 17-ketosteroid excretion lower during the bleeding phase than during the interval phase of the cycle (3). In the establishment of normal values, it has been our practice to measure consecutive 24 hour specimens of urine during the whole interval between menstrual flowings and to report the average of these individual values.

TABLE 3. URINARY EXCRETORY VALUES FOR ANDROGENS AND 17-KETOSTEROIDS OF WOMEN DURING THE REPRODUCTIVE PERIOD

Investigators	Androsterone	
	mg./24 hr.	I.U./*24 hr.*
Bioassay of androgens		
Simpson, et al. (34)		.0–.50[1]
Koch (16)		30–100
Gallagher, et al. (17)		42– 56
Dingemanse, et al. (10)		30– 60
Callow, et al. (14)		5– 60
Glass, Bergman (80)		20– 40
Holtorff, Koch (24)	4.5– 8.5	45– 85
Greene (81)	2.5–12.5	25– 12.5
Colorimetric assay of 17-ketosteroids		
Chou, Wu (19)	1.4– 6.5	14–.65
Callow, et al. (14)	1.7–12.6	17–126
Hamblen, et al. (6)	1.5– 4.7	15– 47
Friedgood, Whidden (75)	4.3–17.3	4–173
Drips, Osterberg (82)	2.5–16.1	25–161
Baumann, Metzger (42)	3.8– 5.0	38– 50
Holtorff, Koch (24)	21.7–47.3	217–473
Talbot, et al. (23)	6.5–17.4	65–174
Fraser, et al. (83)	5.1–14.2	51–142
Werner (84)	5.4–19.0	54–196

[1] Capon units.

METHODS OF STUDY

Consecutive 24-hour specimens of urine were collected for the measurement of 17-ketosteroids. All urine was collected during the non-bleeding phases of the menstrual cycle. The urine was refrigerated during the time of its collection and until its examination.

Prior to assay, hydrolysis and extraction of the urinary specimens were carried out by the technic of Cuyler and Baptist, previously described in detail (85). A stage in this technic comprised repeated washings of the extracts with alkali solutions to remove estrone, itself a 17-ketosteroid.

Colorimetric estimations of 17-ketosteroids were made by the method of Oesting (86, 87) using the Oesting-Hellige colorimeter. Oesting's method of calculation of results was employed and, following his system, amounts of 17-ketosteroids have been expressed in terms of international units of androsterone. In doing so, we have been fully cognizant of the fact that the data obtained did not permit any such interpretations as to the bioactivity or the chemical identity of the substances estimated. This practice represented the acceptance of an arbitrary 'measuring stick,' similar to 'color-units of sterone.' Results expressed in I.U. of androsterone may be converted directly to those expressed as mg. of crystalline androsterone by division by 10.

As previous considerations have indicated, our method of hydrolysis, extraction and purification has resulted in colorimetric data which have been consistently lower than those others have reported. In interpreting our results, comparisons should be made with the range of excretion established by us for healthy women with normal ovarian function. This daily range is equivalent to 15 to 47 I.U. of androsterone.

All patients whose urinary titers were investigated had received routine medical, gynecologic and endocrine surveys. All had *B.M.R.* determinations and roentgenograms of the sella turcica.

CLINICAL DATA

A total of 34 women, whose ages ranged from 17 to 48 years, and who presented either diverse grades

TABLE 4. CLINICAL DATA ON PATIENTS HAVING OVARIAN FAILURE ASSOCIATED WITH HIRSUTISM AND VIRILIZING SYNDROMES[1]

	Patient	Age	Distribution of hirsutism	Escutcheon	Enlarged clitoris	Hypertension	B.M.R.	Distribution of obesity	Menstrual irregularities	Daily 17-Ketosteroid Excretion		
										Range	Average	No. of det.
		yr.					%			I.U.	I.U.	
Hirsutism with diabetes mellitus	JJ	37	Face				+ 9	Thorax; abdominal apron	Menometrorrhagia	16– 81	44	13
	LC	39	Face	♂						48– 52	50	4
	LR	48	Face; thighs	♂	+	+	+ 3			14– 84	54	6
Hirsutism not associated with obvious endocrine disease	JY	17	Face; areolae; abdomen	♂			+ 5	Symmetrical	Oligomenorrhea	76–105	90	7
	VD	20	Face; areolae; arms				+ 1			44– 86	62	6
	JP	21	Face; lower extremities				−26		Metrorrhagia	45– 88	69	9
	SJ	21	Face; abdomen				− 1			63–135	114	11
	DM	21	Upper lip; areolae				− 5	Symmetrical		81–180	138	14
	DW	23	Face; areolae				−10			43–166	93	17
	RG	23	Face; areolae; arms; abdomen				± 0		Oligomenorrhea	72–130	104	8
	FN	26	Face; lower extremities			+	+ 2	Buffalo	Oligomenorrhea	14– 88	51	15
	EE	26	Face; areolae; lower extremities	♂			−12	Generalized	Hypomenorrhea	25–121	71	15
	MF	26	Entire body				−11		Oligomenorrhea	66–120	91	12
	FC	26	Chin; neck; abdomen				− 8	Symmetrical		72–130	94	7
	EH	27	Face; lower extremities	♂			− 6		Polymenorrhea	33– 78	60	18
	MB	28	Face; areolae; arms				+ 4			16– 81	38	18
	WB	30	Face	♂	+		−17			18– 65	32	10
	VI	34	Upper lip; lower legs				+ 1	Abdominal apron	Oligomenorrhea	69–128	109	14
Presumed adrenogenital disorder	PW	20	Face; breasts; abdomen; legs	♂	+	+	+ 6		Oligomenorrhea	41–111	95	9
	MB	20	Face	♂			−17	Abdominal apron	Oligomenorrhea	23–180	118	5
	EW	21	Entire body					Girdle		43– 90	64	17
	MS	25	Abdomen; lower extremities	♂	+		+11	Girdle	Amenorrhea	54– 68	59	8
	SJ	27	Face; abdomen; lower extremities		+		− 4			66– 84	73	3
	JC	29	Face; arms; lower extremities		+					47–119	86	7
	LM	30	Face; thorax; abdomen		+		+ 4	Buffalo	Menorrhagia	16– 90	53	10
	AD	31				+	− 6	Buffalo	Hypomenorrhea	10–265	118	20
	IL	32	Face; thorax; abdomen; extremities	♂		+	± 0	Buffalo	Oligomenorrhea	25–104	62	22
	WH	32	Face; abdomen		+		−20			24–178	63	14
	LC	32	Face; lower extremities	♂	+	+	+12	Generalized	Oligomenorrhea	33–189	70	14
	AT	37	Face; thorax; abdomen	♂			−15			29– 91	66	7
	BR	38	Abdomen; lower extremities			+			Hypomenorrhea	33– 87	51	5
Adrenal hyperplasia, verified	IB	20	Face; abdomen; extremities	♂	[2]	+	+ 3		Delayed menarche	108–265	199	7
	HG	23	Face; lower extremities	♂	+	+	+ 7	Generalized	Oligomenorrhea	9– 95	34	19
	EM	27	Entire body	♂	+		−15		Delayed menarche	87–389	228	10

[1] Blank spaces in table represent negative findings.
[2] Clitoris amputated.

of hirsutism or frank virilizing syndromes associated with primary or secondary hypoovarianism, was studied.

These patients fell into 4 clinical groups dependent upon their symptomatology and physical findings.

Group 1, 3 patients with hirsutism and diabetes mellitus

Group 2, 15 patients with hirsutism which was not associated with any obvious endocrine disease

Group 3, 13 patients with presumed adrenogenitalism

Group 4, 3 patients with adrenal cortical hyperplasia verified at operation

TABLE 5. SUMMARY OF THE CLINICAL DATA ON THE PATIENT GROUPS IN TABLE 4

	No. of Patients	Age range, av. age	Predominant distribution of hirsutism	Escutcheon		Enlarged clitoris	Hypertension	Daily 17-Ketosteroid Excretion		
				♀	♂			Range of	Mean	No. of det.
								I.U.	I.U.	
Hirsutism with diabetes mellitus	3	37–48 41	Face (chin)	1	2	1	1	44– 54	49	23
Hirsutism not associated with obvious endocrine disease	15	21–34 24.6	Face; thorax; abdomen; extremities	11	4	1	1	32–138	81	181
Presumed adrenogenital disorder	13	20–38 28.7	Face; thorax; abdomen; lower extremities	7	6	7	5	51–158	78	141
Adrenal cortical hyperplasia[1]	3	20–27 23.3	Face; abdomen; lower extremities		3	2[2]	2	34–228	154	36

[1] Verified at operation.
[2] Clitoris of one patient had been amputated.

TABLE 6. REPORTED VALUES OF URINARY ANDROGENS AND 17-KETOSTEROIDS IN WOMEN WITH HIRSUTISM AND VIRILIZING SYNDROMES

Diagnosis	Investigator	No. Patients	Assay, as Androsterone		
			Bioassay, androgens	Colorometric,	17-ketosteroids
			I.U./24 hr.	mg./24 hr.	I.U./24 hr.
Hirsutism, virilism or adrenogenital disorder.	Simpson et al. (34)	6	30–180[1]		
	Koch (27)	13	5– 55		
Adrenal or pituitary pathology not established	Callow et al. (20)	8	3– 93		
	Glass, Bergman (80)	10	30– 92		
	Crooke, Callow (40)	1	12		
	Dorfman et al. (88)	7	18– 51		
	Holtorff, Koch (24)	2	6–150		
	Baumann, Metzger (42)	2	6.5–7.7		
	Greene (81)	1	190		
	Callow et al. (20)	8		4.8–23.3	48–233
	Crooke, Callow (40)	26		14 within normal range; 12 from normal to 3×normal range.	
	Friedgood, Whidden (75)	21		2.8 –48.8	28–488
	Drips, Osterberg (82)	11		3.19–14.3	32–143
	Talbot et al. (23)	1		30	300
	Holtorff, Koch (24)	2		15.0, 15.6	150, 156
	Baumann, Metzger (42)	2		9.3, 15.3	93, 153
Cushing's Disease					
a) Adrenal or pituitary pathology not established	Simpson et al. (34)	2	50, 90[1]		
	Callow (12)	13	10–119		
	Drips, Osterberg (82)	3		0.0– 5.0	0– 50
	Talbot et al. (23)	2		11.2–14.8	112–148
	Fraser et al. (83)	2		7.5–36.0	75–360
b) Basophilic adenoma of pituitary	Fraser et al. (83)	1		11.5	115
c) Adrenal cortical hyperplasia+basophilic adenoma of pituitary	Crooke, Callow (40)	1	6	0.0	0.0
d) Absence of adrenal pathology verified at operation	Crooke, Callow (40)	1		17.8	178
e) Adrenal cortical hyperplasia	Fraser et al. (83)	1		7.6–13.8	76–138
f) Adrenal cortical carcinoma	Simpson et al. (34)	1	200–400		
	Crooke, Callow (40)	1	2200		
	Crooke, Callow (40)	2		1) 20–35×normal 2) <normal	
	Fraser et al. (83)	2		19.9, 74	199, 740
Adrenal cortical pathology, nature not specified	Koch (27)	2	0, 44		
Adrenal cortical hyperplasia	Simpson et al. (34)	1	80–300[1]		
	Dorfman et al. (88)	1	140		
Adrenal cortical adenoma	Lukens, Palmer (89)	1	160		
Adrenal cortical carcinoma	Slot (37)	1	2200		
	Koch (27)	2	69, 480		
	Dingemanse et al. (35, 36)	3	600, 2200		
	Callow (12)	2	320, 3500		
	Friedgood, Gargill (41)	1	625, 842		
	Baumann, Metzger (42)	1	32.1		
	Friedgood, Whidden (22)	2		45, 325	450, 3250
	Friedgood, Whidden (75)	2		36.5, 126.5	365, 1265
	Talbot et al. (23)	2		160, 166	1600, 1660
	Baumann, Metzger (42)	1		368	3680
Adrenal cortical tumor, nature not specified	Fraser et al. (83)	1		132–220	1320–2200

TABLE 6.—*Continued*

Diagnosis	Investigator	No. Patients	Assay, as Androsterone		
			Bioassay, androgens	Colorimetric,	17-ketosteroids
			I.U./24 hr.	mg./24 hr.	I.U./24 hr.
Adrenogenital disorder; adrenal cortical hyperplasia	Talbot et al. (23)	2		29, 60	290, 600
	Fraser et al. (83)	3		8.2–82.6	82– 826
Arrhenoblastoma of ovary	Fraser et al. (83)	1		5.3– 8.5	53– 85
Disgerminoma with hirsutism	Fraser et al. (83)	1		7.1– 9.0	71– 90
Masculinoblastoma	Rottino, McGrath (90)	1		15.7–17.3	
Achard-Thiers' syndrome	Simpson et al. (34)	1		<80[1]	

[1] These values are capon units.

The number of 24-hour specimens of urine examined per patient ranged from 3 to 20. A total of 381 specimens was studied, an average of 11.2 per patient.

The pertinent clinical and hormonal data of these individual patients are summarized in table 4. These data on the 4 groups of patients are summarized in table 5.

None of these patients yielded any abnormal findings upon roentgenologic studies of the sella turcica. One of the patients (*I.B.*) of the verified adrenal hyperplasia group had had a unilateral adrenalectomy 5 years prior to the initiation of the studies reported by us. Her visit to the Endocrine Division was occasioned by increasing symptoms and signs of virilization. The other 2 patients of this group had exploratory laparotomies and unilateral adrenalectomies at Duke Hospital following their examinations in the Endocrine Division.

DISCUSSION

Our data are perhaps more consistent in indicating the occurrence of moderately elevated titers of 17-ketosteroids in association with simple hirsutism and virilizing syndromes than most of those of other workers. Striking and unexplainable, individual exceptions, however, have been encountered by us. Even those values established by various workers for urinary androgens under these circumstances have shown inconsistencies similar to those for 17-ketosteroids and have been generally of much smaller amounts. The only consistently and inordinately increased values for 17-ketosteroids and androgens have been found in adrenal cortical carcinomata. No patients of this nature are included in our studies. Table 6 summarizes the findings of various workers in regard to the urinary excretion of androgens and 17-ketosteroids under the various conditions considered in this communication. Due to the various definitions of normal ranges of excretion dependent upon the technics of assay employed, the data in this table should be correlated with those in table 3, which gives a summary of these.

Only 4 of the 34 patients studied by us excreted quantities of 17-ketosteroids which fall within the normal range. Nearly 59% of the patients, or 20, showed, however, only moderately elevated titers. These ranged from the upper limit of normal to twice this limit (100% increase). Of these 20 patients, 4 had titers which were only 20% increased over the upper limit of normal. Ten patients, or approximately 29.5% of the group, however, excreted 17-ketosteroids in amounts greater than twice that of the upper limit of normal range. The titers of these 10 patients ranged from 84 I.U. to 228 I.U., representing from 100 to 400% increases.

These data do not differ in kind from those obtained upon women being treated with intramuscular injections of testosterone propionate, given in doses ranging from 10 to 25 mg. and at intervals of 2 days. Studies of the urinary excretion of 17-ketosteroids of these women before and during therapy indicated that the excretory values of 17-ketosteroids were elevated by amounts which were equivalent to increases of 18% to 300% over the pretreatment levels of excretion (2). Ample clinical evidence has been submitted in the past to warrant the conclusion that therapy of this order if persistently carried out will produce hirsutism and virilization. Therefore, it is obvious that the excretion of 17-ketosteroids in hirsute or virilizing conditions of spontaneous origin does not differ markedly from that in patients with inadvertently induced andromimetic responses.

In general there was a tendency for the elevation in 17-ketosteroids values to parallel the degree of hirsutism or virilization. There were exceptions, including *H.G.*, a patient with verified adrenal cortical hyperplasia. The group composed of patients with verified adrenal cortical hyperplasia yielded the highest titers. A number of the patients with hirsutism, not associated with any obvious endocrine

disease and some of those patients in the group with presumed adrenogenital disorders yielded only moderately elevated titers. On the other hand, there were a number of patients in this latter group who had sufficient clinical evidence of androgenic adrenal hyperactivity to have warranted exploratory laparotomy. The 3 patients with diabetes and slight hirsutism, but not in any way typical of 'diabète des femmes à barbé' of Achard and Thiers, had practically normal titers. Some workers (83) have found titers of 17-ketosteroid somewhat depressed in diabetes, probably due to the undernutrition and metabolic disturbance. Such an explanation may be offered here.

To us, at present, the chief interest of these observations on the excretion values of 17-ketosteroids in hirsutism and virilizing syndromes lies in the fact that they concern patients in whom, by fact or by theory, the primary endocrine disturbance is hyperactivity of the adrenal cortex and the secondary endocrine disturbance is hypofunction of the ovaries. These data, representing, therefore, potential measurements of varying degrees of primary hyperactivity of the adrenal, are to be compared with similar data submitted in the next 3 papers in which the endocrine disturbance of patients is primarily ovarian. This comparison is for the purpose of testing a theory previously expressed by members of our group (2) that a balance exists between estrogenic ovarian and androgenic adrenal functions (or between the gonado-pituitary and adreno-pituitary axes), which is upset by ovarian failure in the direction of increased adrenal activity. The data of the present paper can serve as a valuable 'measuring stick' as to the quantitative expectations in urinary 17-ketosteroid values when the adrenal cortex becomes hyperactive.

SUMMARY

The urinary excretion of 17-ketosteroids was investigated in 34 women, whose ages ranged from 17 to 48 years and whose symptomatology of adrenal hyperactivity ranged from simple hirsutism of moderate degree to states of marked virilization. The values established for urinary 17-ketosteroids varied from normal ones to ones increased 400% over normal. These data are regarded as comparable to those obtained after repeated injections at 2-day intervals of moderate doses (10 to 25 mg.) of testosterone propionate. The data, obtained from the present study, are to be accepted as 'measuring sticks' of primary adrenal hyperactivity and are to be employed in subsequent studies of adrenal function as influenced secondarily by diverse grades of ovarian failure.

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Urinary Excretion of 17-Ketosteroids in Ovarian Failure

II. In Menometrorrhagia[1]

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Patients who experience too frequent or excessive uterine bleeding of functional origin may have diverse grades of ovarian failure. The severity of their ovarian disturbances can be gauged roughly by studies of endometrial biopsies taken at the onset of episodes of bleeding. Data from these studies as well as from a mass of clinical observations support the point of view that few of these patients exhibit any objective evidences of basic estrogenic deficiency, although intercurrent episodes of relative estrogenic deficiency obviously account for their menometrorrhagia (1). As a rule, these individuals are well feminized, lack the endocrinopathic signs of glandular diseases and are not possessed of hypoplastic sexual organs. Their ovarian disturbance concerns progesterone rather than the estrogens, being characterized by minimal or no corpus luteum activity. The proper selection of a group of patients with functional menometrorrhagia, characterized endocrinologically by deficiencies in corpus luteum function and not by any gross disturbances in estrogenic levels, permits study of the influence of simple corpus luteum failure of the ovaries upon the urinary excretion of 17-ketosteroids, i.e., androgenic function of the adrenals. The present study has this purpose in mind. The next two studies will evaluate the effects of estrogenic ovarian failure upon the urinary excretion of 17-ketosteroids.

METHOD OF STUDY

The methods for the hydrolysis and extraction of urine specimens and for the colorimetric estimation of 17-ketosteroids, described in the first paper of this series, were employed in the present studies. Twenty-four-hour urine specimens, collected during nonbleeding phases of the cycle, were quantitated.

All of the patients investigated received routine medical, gynecologic and endocrine surveys. All had determinations of B.M.R. and roentgenograms of the sella turcica.

Repeated endometrial biopsies were made at the onset of episodes of uterine bleeding (within the first 12 to 18 hours after the beginning of flowing) and the tissue obtained was studied by one of us (E. C. H.) as an index to the nature and severity of ovarian disturbance. A number of other special studies was done including quantitations of the urinary pregnandiol complex; these data are not included in the present report.

CLINICAL DATA

A total of 10 women, whose ages ranged from 16 to 38 years and who presented the common symptom of excessive or too frequent uterine bleeding, were studied. None of these patients exhibited any signs or symptoms of endocrine disease except the menstrual irregularity. All of the studies reported were made prior to initiation of any therapeutic measures.

The number of consecutive 24-hour specimens of urine examined per patient ranged from 5 to 24. A total of 145 specimens was studied, an average of 14.5 per patient.

Table 1 summarizes the clinical data on these 10 patients, which include B.M.R., titers of urinary 17-ketosteroids and endometrial responses.

Roentgenologic studies of the sella turcica in no instance revealed any pathology.

DISCUSSION

The urinary titers of 17-ketosteroids of these patients fell within the range established for healthy cyclic women with normal ovarian function, except in two instances wherein the average daily values were 49 I.U. The upper range of our group of normals is 47 I.U. For all purposes, we consider the titers of all

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[1] See footnotes to the first of this series of communications.

TABLE 1. SUMMARY OF CLINICAL DATA ON PATIENTS HAVING OVARIAN FAILURE ASSOCIATED WITH MENOMETRORRHAGIA

Patient	Age	Menstrual history and duration or irregularity	Endometrium at bleeding, before treatment	Daily 17-Ketosteroid Excretion			
				B.M.R.	Average, before treatment	Range	No. of det.
	yr.			%	I.U.	I.U.	
HE	18	polymenorrhea since menarche (4 yr.)	progestational	−14	33	12–52	14
CW	19	menorrhagia since menarche (5 yr.)	progestational	− 7	49	24–97	15
BB	18	metrorrhagia 1 yr.	mixed progestational	+ 8	43	24–62	8
OU	28	menorrhagia since menarche (15 yr.)	mixed progestational	+ 7	41	10–56	21
EG	32	menorrhagia 18 yr.	mixed progestational	−21	49	39–70	5
MS	38	polymenorrhea 10 yr.	mixed progestational	+ 5	16	10–23	14
CB	17	polymenorrhea and hypermenorrhea 2 yr.	persistent estrogenic	− 3	34	17–49	11
NW	25	polymenorrhea and hypermenorrhea since menarche (12 yr.)	persistent estrogenic	− 6	32	13–52	18
AB	16	menorrhagia since menarche (3 yr.)	hyperestrogenic	−11	35	20–58	24
VH	29	Oligomenorrhea, menorrhagia 13 yr.	hyperestrogenic	− 7	25	12–45	15
Av.	24				35.6		total 145

10 patients to be normal. These data bear out the impression gained by some members of our group (2, 3) from a much smaller group of patients that no significant alterations in 17-ketosteroid excretion from normal ranges occurred in functional menometrorrhagia.

These findings are taken to indicate that diverse grades of corpus luteum hormone function, ranging from normal to the absence of progestin secretion, *per se* have no effects upon the level of 17-ketosteroid excretion. This fact is taken to be evidence that progestin does not enter into any close reciprocities with the adrenal androgens, i.e., that no progestin: androgen ratio exists. A corollary to these statements seems justified to the effect that simple corpus luteum failure of the ovaries is followed by no secondary hyperactivity of the adrenal cortex.

The data presented in this report and those to be presented in the next studies indicate that the generally made statement to the effect that the hormonologic associations of all of the functional irregularities of uterine bleeding may be the same is probably not true. Therapeutic experiences also have been against such a general statement of fact, that is, patients with functional menometrorrhagia yield a much better salvage of ovarian function than those with amenorrhea (4).

SUMMARY

The urinary excretion of 17-ketosteroids was investigated in 10 women, whose ages ranged from 16 to 38 years and whose common symptom was menometrorrhagia, related to simple corpus luteum failure of the ovaries. The values for urinary 17-ketosteroids of these women did not differ from those of healthy cyclic women with normal ovarian function. It is inferred from these studies that progesterone plays no active part in the reciprocities of function of the gonado-pituitary and the adreno-pituitary axes.

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Urinary Excretion of 17-Ketosteroids in Ovarian Failure

III. In Amenorrhea[1]

[17-Ketosteroids]

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Amenorrhea when due to disturbed ovarian function is associated commonly with signs of estrogenic deficiency, among which are hypoplasias or regressions in the sexual system, including atrophic alterations in the endometrium and vaginal mucosa. These objective manifestations of estrogenic deficiency are evidenced as failures of sexual maturation in adolescent hypoövarianism, and as sexual regressions in intercurrent hypoövarianism of the adult. As a rule, in adult hypoövarianism the degree of sexual regression increases with the duration of the amenorrhea. Patients with adolescent and adult hypoövarianism, as characterized by delayed menarche or intercurrent amenorrhea, present the opportunity of investigating the influences of estrogenic deficiencies upon the urinary excretion of 17-ketosteroids, that is, the androgenic activity of the adrenals. The present study and the one to follow have this purpose in mind.

METHOD OF STUDY

The methods for the hydrolysis and extraction of urine specimens and for the colorimetric estimation of 17-ketosteroids, described in the first paper of this series, were employed in the present study. Consecutive 24-hour specimens of urine were employed.

All the patients investigated received routine medical, gynecologic and endocrine surveys. All had determinations of B.M.R. and roentgenograms of the sella turcica.

The majority had serial endometrial biopsies. The endometrial tissue obtained was studied by one of us (E. C. H.) as an index to the degree of the ovarian failure. A number had studies of urinary pregnandiol-complex titers. The patients with adolescent ovarian failure had roentgenologic evaluations of osseous age. In certain instances, subsequent clinical responses of many of these patients to therapeutic endeavors, especially those employing gonadotropins and thyroid substance, afforded confirmatory data on the cause of the ovarian failure.

CLINICAL DATA

A total of 31 patients with amenorrhea was studied. *Group 1* is comprised of 8 women, whose ages ranged from 15 to 26 years, and whose delayed menarches were related either to hypoantuitarism, intrinsic hypoövarianism, or to faulty somatic development of the uterus. All of the patients of this group, except the one whose amenorrhea was related to faulty development of the uterus, exhibited no or only partial sexual maturation. *Group 2* is comprised of 23 women, whose ages ranged from 16 to 38 years and whose episodes of intercurrent amenorrhea had lasted from 2 months to 5.5 years. The majority of the patients whose episodes of amenorrhea had lasted 6 months or more, exhibited some signs of regressive alterations in their genital systems.

The number of 24-hour specimens of urine examined per patient varied from 2 to 37. The total number of specimens quantitated was 459, an average per patient of 14.8.

Roentgenologic studies of the sella turcica yielded no significant pathologic findings.

Table 1 summarizes the clinical data on the patients with delayed menarche with particular regard to B.M.R. and titers of urinary 17-ketosteroids. Table 2 gives similar data on the patients with intercurrent amenorrhea.

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TABLE 1. SUMMARY OF CLINICAL DATA ON PATIENTS WITH DELAYED MENARCHE RELATED TO HYPOANTUITARISM, INTRINSIC OVARIAN FAILURE, OR FAULTY SOMATIC DEVELOPMENT OF THE UTERUS

Patient	Age	Diagnosis	B.M.R.	Daily 17-Ketosteroid Excretion		
				Range	Average	No. of det.
	yr.		%	I.U.	I.U.	
MP	15	hypoantuitarism	+10	10–43	19	19
MJ	17	hypoantuitarism	−13	16–54	30	9
SE	19	hypoantuitarism	− 6	11–60	29	36
NT	24	hypoantuitarism		15–30	23	10
HK	24	hypoantuitarism	−12	12–34	21	9
ER	19	intrinsic ovarian failure	− 3	13–25	18	14
MKB	26	intrinsic ovarian failure	−13	17–43	32	10
LFB	16	congenital absence? of uterus	−10	51–252	145	12

Endometrial responses are not reported in detail; in those instances in which they were done, inadequate estrogenic proliferation was found generally if the amenorrhea had lasted 6 months or more.

Values of urinary pregnandiol-complex, when investigated, were negative ones in the group of patients with delayed menarche and in those whose intercurrent amenorrhea had lasted 6 months or more.

Patients, whose amenorrhea had lasted less than 6 months, were found on some occasions to excrete small amounts of pregnandiol-complex and to have endometria in good states of estrogenic proliferation. These observations cast doubt upon the advisability of assuming the existence of more than oligomenorrhea as the menstrual irregularity of these patients.

DISCUSSION

The urinary excretion of 17-ketosteroids is uniformly different in the patients of the two groups, presented in tables 1 and 2. Patients with delayed menarche, associated with delayed or incomplete sexual maturation, excreted 17-ketosteroids in amounts which fell either within the lower range for healthy cyclic women with unimpaired ovarian function, or slightly below the lower limits of this range, that is, 15 to 47 I.U. As a matter of fact, 6 of the 8 patients excreted 17-ketosteroids in amounts considered lower than the normal limit.

Two explanations are offered in regard to these findings. The low excretionary values of 17-ketosteroids may be correlated with the deficient somatic

TABLE 2. SUMMARY OF CLINICAL DATA ON PATIENTS WITH INTERCURRENT AMENORRHEA

Patient	Age	Duration of Symptoms	B.M.R.	Daily 17-Ketosteroid Excretion			
				Range	Average	No. det.	Mean for group
	yr.	*months*	%	I.U.	I.U.		
NP	17	2		36–126	77	19	
AW	25	2	+ 1	11– 57	31	26	
AS	22	3	−22	16– 29	21	14	41
SB	22	3	− 6	14– 56	35	14	
LM	19	4		27– 63	48	12	
RP	26	5	−18	25– 48	33	11	
NAP	27	6	−16	16–111	49	6	
BP	28	6	+ 3	33– 97	68	14	
LAM	17	7	− 3	42– 81	63	12	
HR	23	7	− 1	52– 83	69	14	
CS	22	8	+ 3	11– 53	27	10	
LB	23	8	− 6	13– 47	33	14	60
IG	29	8	+ 4	73–108	93	8	
CW	21	8	−13	85–135	97	2	
ML	26	9	− 6	10– 57	30	37	
DS	24	10	− 4	22–157	61	14	
LR	16	11	−15	11–117	71	20	
CE	18	12	−12	32–114	56	16	
JE	30	18	− 4	22–148	69	28	
SS	30	24	− 8	47–178	93	7	84
JW	26	24	−13	22–159	101	13	
AC	38	36	− 2	55–122	88	12	
FB	29	66	−15	50–151	95	17	

and sexual development of these patients; such observations have been made in the instances of pre-adolescents (1, 2). The other explanation is that hyperactivity of the adrenal cortex, i.e., increased excretion of 17-ketosteroids, only occurs after adult or late ovarian failure. The sympathies of the gonado-pituitary and the adreno-pituitary axes apparently develop only after adult functions of the gonado-pituitary axis have been established. The superimposition of the reciprocities of the gonado-pituitary axis of the sexually adequate adult upon the primordial adreno-pituitary axis initiates reciprocities, which when disturbed by late ovarian failure result in compensatory hyperactivity of the adrenal cortex.

Patients with intercurrent amenorrhea of greater than 5 months' duration show quite consistently increased urinary excretions of 17-ketosteroids. Of the 6 patients whose amenorrhea had lasted from 2 to 5 months, only one excreted more than normal amounts of 17-ketosteroids. Strictly speaking, one is not privileged to refer to the absence of menses for 2–3 months as amenorrhea, but should rather use the term oligomenorrhea, which indicates infrequent but recurring flowings. All of the patients of the first category may have been of this type.

The 17 patients of the remaining two categories of *group 2*, that is, those with amenorrhea of 6 to 11 months' duration, and of 12 months to 5.5 years' duration, showed with 3 exceptions definite increases in their urinary values of 17-ketosteroids. When the two categories are considered as units rather than from the standpoint of the individual patients comprising them, there is an increase of 40% in the 17-ketosteroid titers of the patients with amenorrhea of more than 1 year in duration over those whose amenorrhea had lasted 6 months to 1 year.

These findings confirm the impressions gained by us from the study of a smaller group of patients, the results of which have been reported (3, 4).

These increases in 17-ketosteroid titers in patients with adult ovarian failure of estrogenic type are related to compensatory and secondary adrenal hyperfunction. These data do not permit any assumptions as to the duration of this secondary adrenal hyperactivity.

The effects of intercurrent estrogenic failure of the ovaries, upon 17-ketosteroid excretion, i.e., adrenal function, contrast strikingly with the absence of any effects in regard to patients with simple corpus luteum failure of the ovaries. They bespeak a fundamental difference in the basic hormonology of the two groups of patients.

SUMMARY

The urinary excretion of 17-ketosteroids was investigated in 8 patients, aged from 15 to 26 years, with delayed menarche and in 23 patients, aged 16 to 38 years, with intercurrent amenorrhea of durations which ranged from 2 months to 5.5 years. The majority of these patients exhibited physical signs of estrogenic deficiency. There was no elevation of the 17-ketosteroid titers in the group of patients with delayed menarche while, on the other hand, definite elevations in the titers occurred in the patients with intercurrent amenorrhea which had lasted 6 months or more. A correlation apparently existed between the duration of the amenorrhea and the amount of 17-ketosteroid excretion. These findings indicate that late or intercurrent estrogenic ovarian failure precipitates androgenic hyperfunction of the adrenal cortex.

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Urinary Excretion of 17-Ketosteroids in Ovarian Failure

IV. During the Climacteric and after Artificial Menopause[1]

[17-Ketosteroids]

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Estrogenic ovarian failure, more severe in degree than that which occurs in many instances of intercurrent amenorrhea during the reproductive period, occurs physiologically during the sexual aging of woman, or may be induced by radium or roentgenologic therapy. Complete ovarian deficiency occurs in bilaterally oöphorectomized women. In all of these instances, the causes of the failure may be reduced to a simple common denominator, intrinsic ovarian damage, resulting from senility, radium, roentgen-ray or surgery. Patients with this type of intrinsic ovarian failure afford an excellent opportunity for the investigation of possible alterations in the androgenic functions of the adrenals secondary to ovarian deficiency or deprivation. If the findings, reported in the previous communication upon patients with amenorrhea, were interpreted correctly, studies of 17-ketosteroid excretion during the climacteric and after artificial menopause should show similar, if not more marked, elevations than those during amenorrhea. The present communication reports a study of 17-ketosteroid excretion in women during the climacteric and after artificial menopause.

METHOD OF STUDY

The methods for the hydrolysis and extraction of urine specimens and for the colorimetric estimation of 17-ketosteroids, described in the first paper of this series, were employed in the present study. Consecutive 24-hour specimens of urine were quantitated.

All of the patients investigated received routine medical, gynecologic and endocrine surveys. All had determinations of B.M.R. and roentgenograms of the sella turcica.

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[1] See footnotes to the first of this series of communications.

CLINICAL DATA

A total of 41 women was studied. These patients were segregated into 4 groups according to the exciting cause and degree of their ovarian failure.

Group 1 comprised 12 women, aged 35 to 51 years, who, because of symptoms of presumed spontaneous estrogenic deficiency, were judged to be experiencing the climacteric. None of this group had ceased to menstruate.

Group 2 was composed of 5 women, aged 39 to 45 years, who had had individual episodes of amenorrhea ranging in duration from 3 months to 3 years, as a result of the intrauterine application of radium or roentgenologic therapy of the ovaries.

Group 3 consisted of 12 women, aged 21 to 48 years, who had had bilateral oöphorectomies at diverse times, which ranged from 9 days to 16 years prior to that of the present study.

Group 4 was made up of 12 women, aged 42 to 74 years, who had experienced physiologic menopause from 5 months to 26 years prior to the time of the present study.

The number of consecutive 24-hour specimens of urine examined per patient ranged from 4 to 26. A total of 528 specimens was studied, an average of approximately 12.9 per patient.

Table 1 presents a summary of the clinical data on these 41 patients.

No pertinent correlations were established from the studies of the B.M.R. and roentgenologic examinations of the sella turcica, and, accordingly, these data are omitted from this report.

DISCUSSION

The data on *group 1*, comprised of 12 women who presumably were experiencing climacteric hypo-

TABLE 1. SUMMARY OF CLINICAL DATA OBTAINED ON PATIENTS DURING THE CLIMACTERIC OR ARTIFICIAL MENOPAUSE

Exciting Cause of ovarian failure	Patient	Age	Duration of menopause	Daily 17-Ketosteroid Excretion				
				Range	Average	No. of det.	Mean for subgroup and group	
		yr.		I.U.	I.U.		I.U.	I.U.
Group 1. Physiologic climacteric, premenopausal stage	EW	35		54–100	78	14		
Subgroup 1	CL	37		41–122	81	9		
	JH	38		54–202	121	21		
	LR	39		45–144	85	10		
	IH	40		38–208	77	14		
	LB	44		29–130	97	18		
	JL	44		57–114	75	10	88	
Subgroup 2	FL	46		36– 45	39	6		
	MC	48		29– 60	40	11		
	BC	50		20– 63	41	16		
	NW	51		32– 94	58	12		
	EM	51		22– 57	36	14	43	69
Group 2. Radium or Roentgen Therapy	MW	40	3 mo.	29– 72	45	14		
	CR	40	3 mo.	15– 85	39	10		
	LP	45	4 mo.	39– 84	55	12		
	FT	39	32 mo.	32–114	68	14		
	FG	39	36 mo.	24– 72	38	14		49
Group 3. Bilateral Oöphorectomy	VIII	30	9 days	10– 25	15	7		
Subgroup 1	IX	35	11 days	10– 18	13	5		
	X	37	12 days	12– 54	24	8		
	XI	41	12 days	16– 58	35	10		
	XII	44	24 days	17– 61	31	18	24	
Subgroup 2	MA	34	7 mo.	38– 79	56	12		
	ET	32	3 yr.	32–105	55	10		
	LN	23	5 yr.	51–134	88	10		
	BW	42	7 yr.	55–157	101	11		
	MB	21	8 yr.	28–126	72	23	74	
Subgroup 3	LM	45	16 yr.	27– 91	42	10		
	MS	48	16 yr.	11– 77	38	24	40	48
Group 4. Physiologic Climacteric; Postmenopausal	LF	49	5 mo.	31–157	89	13		
Subgroup 1	NC	48	9 mo.	24–130	63	20		
	NJ	42	9 mo.	22–180	91	11		
	KA	47	12 mo.	21–126	64	26	77	
Subgroup 2	MG	52	2 yr.	73–202	135	12		
	FA	57	2 yr.	72–126	99	4		
	EN	48	2 yr.	60–162	101	7		
	MM	56	4 yr.	53– 74	64	8		
	BC	56	5 yr.	51– 89	64	7	93	
Subgroup 3	HH	62	14 yr.	13– 80	54	15		
	CA	67	25 yr.	16– 47	26	4		
	AM	74	26 yr.	12– 47	36	24	39	72

ovarianism despite the nonoccurrence of actual menopause, indicate that the majority excreted increased amounts of 17-ketosteroids. The averages of daily titers of these patients ranged from 36 to 121 I.U., the average for the group being 69 I.U. Four of the 12 patients, however, excreted normal quantities of 17-ketosteroids. The inclusion in this group of some patients with relatively normal ovarian function, despite their ages or symptomatology, was anticipated, since patients of various ages and presumably at various stages in their premenopausal climacteric processes were chosen deliberately for investigation in the hopes of defining, if possible, the nature of the curve of, and the time of occurrence of any 'peaks' of 17-ketosteroid excretion. The fact that the average 17-ketosteroid excretion of this group was increased by approximately 50% over the upper limit of the range of normal values indicates that some degree of ovarian hypofunction existed in the majority of these women.

The difference in the urinary titers of 17-ketosteroids of the two subgroups of these patients, based on their ages, may be of some significance. The average of the daily 17-ketosteroid excretion of the 7 patients, whose ages ranged from 35 to 44 years, was 88 I.U., while that of the 5 patients, whose ages ranged from

46 to 51 years of age, was 43 I.U. This difference may indicate that age rather than the actual cessation of menstruation is an important factor in the occurrence of compensatory adrenal hyperactivity. Upon this premise, it may be inferred that the excretionary curves of 17-ketosteroids of the older patients had already reached 'peaks' and were, at the time of this investigation, in the process of 'levelling off,' i.e., these patients had made their critical hormonologic adjustments prior their actual menopauses.

The data on *group 2*, composed of 5 women whose episodes of amenorrhea of varying durations were related to previous radium or roentgenologic therapy are consistent with our understanding of the diverse grades of estrogenic ovarian impairment which follow moderate dosages of these therapies. Even much larger doses of radium or roentgenologic therapy than were given these patients cannot be relied upon to produce with certainty absolute hypoövarianism.

Group 3, composed of 12 women, who had absolute ovarian failure due to previous bilateral oöphorectomies, is divided for discussion into 3 subgroups depending upon the recentness of their operative procedures.

Subgroup 1 of *group 3* is made up of 5 patients, whose elapsed times since their operations, as dated from the time of collection of the last 24-hour specimen of urine for study, varied from 9 to 24 days. All of these patients excreted 17-ketosteroids in amounts ranging from 13 to 35 I.U. daily, which were essentially within the ranges established for women with normally functioning, intact ovaries. These data are significant for two reasons. 1) They establish an extra-ovarian source for the 17-ketosteroids under study; 2) they indicate that a latent period exists from the time of occurrence of ovarian failure until such time that evidences of increased adrenal function appear, i.e., increased excretion of 17-ketosteroids. Such a latent period is characteristic of the origin of compensatory hyperactivity and, therefore, good evidence in favor of its occurrence under these circumstances.

Subgroup 2 of *group 3* consists of 5 patients, whose elapsed times since their operation, ranged from 7 months to 8 years. All of these patients excreted increased amounts of 17-ketosteroids, the average daily excretions for different patients ranging from 55 to 101 I.U. The average for the group was 74 I.U. These data indicate the occurrence of definite compensatory hyperactivity of the adrenals and date it as beginning at least within 7 months of oöphorectomy and as continuing for at least 8 years.

Subgroup 3 of *group 3* contains 2 patients, whose elapsed times since their operations were 16 years. Both of these patients excreted normal quantities of 17-ketosteroids, 38, and 42 I.U., per day. These data indicate that compensatory hyperactivity of the adrenals, secondary to ovarian failure, does not continue indefinitely, and that prior to 16 years after oöphorectomy, a 'levelling down' to normal functions occurs.

Some exceptions have been taken by Callow, Callow and Emmens (1) to previous reports of some members of our group (2, 3) of similar findings from studies of a much smaller series of women. These observers, however, admit the possibility that increased urinary excretion of 17-ketosteroids may occur under these circumstances and make the following statement in regard to their own findings: "The results are consistent with the hypothesis that absence of the gonads leads to compensatory activity of the adrenal cortex in some cases, but do not yield positive evidence in its favour."

The urinary excretion of androgens after oöphorectomy has been reported by Dingemanse, Borchardt and Laqueur (4), Callow (5), and Callow, Callow and Emmens (1) as being generally below normal levels. Furuhjelm (6) however has reported that androgenic titers maintain normal levels immediately following oöphorectomy. The urinary excretions of 17-ketosteroid under these conditions have been reported by Callow, Callow and Emmens (1) as being lower generally than the values found in normal women but as having a tendency to overlap normal range. From studies upon a larger series of patients, the same observers, however, have found that the majority of oöphorectomized women excreted normal amounts of 17-ketosteroids although some excreted larger amounts than normal, When, however, the data of these workers are assembled in ascending order dependent upon the duration of time elasped between their operations and the titrations of their urines, a tendency toward increased excretions of 17-ketosteroids, similar to those reported in our studies, is apparent in their findings.

Additional evidence of a different sort as to the ovaries not being the source of formation of the urinary 17-ketosteroids is supplied by Butenandt and associates, Callow and colleagues, and Hirschmann. These workers isolated androsterone (7, 8), dehydroisonadrosterone (1, 8) and etiocholanol-3(α)-17-one (8) from the urine of oöphroectomized women, thus establishing the fact that the same 17-ketosteroids, which occur in the urines of non-gonadectomized males and females occur in the urines of oöphorectomized females.

Resuming the analysis of our data, *group 4*, comprised of 12 women who had experienced physiologic menopauses, is divided for discussion into 3 subgroups depending upon the interval of elapsed time since the cessations of menstruation.

Subgroup 1 of *group 4* is made up of 4 women, whose ages ranged from 42 to 49 years and whose

elapsed times since menopause varied from 5 to 12 months. All of these women excreted 17-ketosteroids in excess of the range established for women with normal ovarian function. Their average daily excretions varied from 63 to 91 I.U., an average for the group of 77 I.U. This latter average represented an increase of almost 64% over the upper value for normal range.

Subgroup 2 of *group 4* is comprised of 5 women, whose ages ranged from 48 to 57 years, and whose elapsed times since menopause varied from 2 to 5 years. All of these women excreted 17-ketosteroids in excess of the range established for women with normal ovarian function. Their average daily excretions varied from 64 to 135 I.U., an average for the group of 93 I.U. This latter average represented an increase of almost 100% over the upper value for normal range.

Subgroup 3 of *group 4* contains 3 women, whose ages ranged from 62 to 74 years, and whose elapsed times since menopause varied from 14 to 26 years. These women excreted essentially normal amounts of 17-ketosteroids. Their average daily excretions varied from 26 to 54 I.U., an average for the group of 39 I.U.

The studies on the 12 postmenopausal women of *group 4*, therefore, yielded data which are in complete agreement with those obtained from the studies on the 12 oöphorectomized women. Physiologic ovarian failure due to sexual senility, accordingly, results in adrenal hyperactivity of the same order as does complete ovarian failure induced by bilateral oöphorectomy. The values for urinary 17-ketosteroids continue to increase after menopause, this increase having begun, as a rule, prior to menopause. The 'peak' of excretion, according to our data, probably occurs generally about 2 years after menopause. There then occurs a gradual 'scaling down' of these excretionary values, although these may remain elevated at least 5 years after the last flowing. During the latter years of life, at least from the 14th to the 26th years after menopause, the values of urinary 17-ketosteroid excretion are of the same order as those of women with normal ovarian function.

Similar data upon a much smaller group of climacteric women suggested to some members of our group an extension of the theory as to the hormonology of the climacteric, which has been reported previously (2, 3). This theory is summarized briefly:

As estrogenic failure of the ovaries occurs, a compensatory hyperactivity of the anterior pituitary ensues which is indicated by an increased gonadotropuria. This increased activity probably involves all the endocrinotropic principles of the anterior pituitary. The adrenal cortex, suffering none of the senile refractivity which occasioned the ovarian failure, responds with an increased steroid output, which results in increases in the urinary excretion of 17-ketosteroids. As the adrenal secretes increased amounts of steroids, the inhibiting influences upon the pituitary, disturbed by decreases in ovarian steroids, are restored and pituitary function tends to return to normal levels. This, in turn, reduces the level of adrenal activity, i.e., decreases the urinary excretion of 17-ketosteroids. There then has been established a stability of th adreno-pituitary axis which characterizes the hormonology of postclimacteric somatic aging. This secondary stabilization of pituitary and adrenal functions probably coincides with the symptomatic stabilization ultimately experienced by climacteric women and it avoids a continuation of hyperpituitary activity until that gland is functionally exhausted. As a corollary to this theory, it was suggested that the adrenals may be considered as functioning as the gonads of the aged and that the sexual regressions which characterize the climacteric have two hormonologic facets: 1), a negative influence, which results from the decreasing levels of estrogens and, 2), a positive influence, which results from the 'defeminizing' or 'ovarian-negating' effects of increasing levels of androgens.

The data reported in the 4 communications of the present series support a broad theory of ovario-adrenal reciprocities, which are mediated chiefly by way of the pituitary. During early life and until adolescence is well advanced, the adrenopituitary axis is dominant in steroid metabolism. When primary or adolescent hypoövarianism occurs and is of sufficiently severe grade to result in failure of sexual maturation, the adreno-pituitary axis retains its dominant position even during adult years. Under these circumstances, the level of adrenal function remains about that characteristic of late preadolescence. Normally, however, with the advent of adolescence and the beginning of full ovarian function, the ovario-pituitary axis is superimposed upon the primordial adreno-pituitary one. The result is that a harmonious, coöperative economy is set up, wherein the pituitary becomes conditioned to working with both adrenals and ovaries and to being influenced, as regards its functions, by the regulatory effects of the intrinsic steroids of both the ovaries and the adrenals. During the reproductive period the ovario-pituitary axis comes to dominate the endocrine system. When, however, ovarian failure occurs from pathologic or physiologic causes, the pituitary, accustomed to being balanced in function by both the adrenal and ovarian steriods, is released in part from its inhibitions and, thereby, becomes hyperactive. Sequentially, the adrenal cortex is stimulated to secrete greater quantities of steroids. The now dominant adreno-pituitary axis is stronger because of its previous conditioning by the ovario-pituitary axis.

Eventually, however, stabilization occurs from the inhibiting effects of the increased amounts of adrenal steroids upon the function of the pituitary. The result is that during the late stages of sexual aging and during somatic senescence, the adreno-pituitary axis becomes stabilized and steroid output reaches normal levels.

The various data tending to support this theory of reciprocities of the adreno-pituitary and ovario-pituitary axes, which have been submitted in this series of communications and in others by members of our group, are summarized:

Adrenal cortical hyperactivity, associated with simple hirsutism or with clear-cut virilizing syndromes, is associated with diverse grades of hypo-ovarianism and is characterized by the urinary excretion of increased amounts of 17-ketosteroids.

Injections of testosterone propionate given to women with essentially normal ovarian function may induce ovarian failure, produce signs of virilization and increase their urinary titers of 17-ketosteroids to about the level of those which characterize spontaneously occurring virilization (2).

The administration of estrogens to women with moderately elevated urinary values for 17-ketosteroids will reduce these values to essentially normal ones (9).

Primary or adolescent hypoövarianism, that is characterized by delayed menarche and incomplete sexual maturation, produces no significant effects upon the urinary excretion of 17-ketosteroids.

Intercurrent or adult estrogenic ovarian failure, characterized by amenorrhea and sexual regressions, is characterized by the urinary excretion of increased amounts of 17-ketosteroids.

Bilateral oöphorectomy has no immediate effects on the urinary titers of 17-ketosteroids. This is proof that the urinary 17-ketosteroids are of extra-ovarian origin, probably adrenal.

After a latent period, the amounts of 17-ketosteroids excreted in the urine of bilaterally oöphorectomized women increase to levels of the same order essentially of those of women with primary adrenal hyperactivity, that is, hirsutism, of moderate degree.

Eventually, following the elapse of a number of years from the time of operation, the urine titers of 17-ketosteroids of oöphorectomized women return to essentially normal levels, that is adrenal function becomes stabilized.

The data on women with ovarian failure due to physiologic climacteric agree qualitatively and quantitatively with those on oöphorectomized women.

Simple corpus luteum failure of the ovaries, as seen in the majority of patients with functional menometrorrhagia, produces no alterations in the urinary values of 17-ketosteroids, that is, progesterone is not involved in the ovario-adrenal reciprocities.

SUMMARY

The urinary excretion of 17-ketosteroids was investigated in 4 groups of women, totaling 41. *Group 1*, 12 women, aged 35 to 51 years, with ovarian failure presumably due to premenopausal stage of the climacteric; *group 2*, 5 women, aged 39 to 45 years, with amenorrhea of relatively short durations, related to previous radium or roentgenologic therapy; *group 3*, 12 women, aged 21 to 48 years, with complete hypo-ovarianism due to bilateral oöphorectomies; and *group 4*, 12 women, aged 42 to 74 years, who had experienced physiologic menopauses.

The majority of the patients of *group 1* excreted increased amounts of 17-ketosteroids. No definite increases in the urinary values of 17-ketosteroids were found in the patients of *group 2*. In *group 3*, the urinary values of 17-ketosteroids were normal in women recently oöphorectomized, that is, 9 to 24 days previously; these were increased definitely in women whose operations had been done from 7 months to 8 years previously; and these titers were normal in 2 women, whose operations had occurred 16 years previously. Quite similar findings were encountered in *group 4*; urinary titers of 17-ketosteroids were increased in those women whose menopauses had occurred from 5 to 12 months and from 2 to 5 years previously, the latter group showing the higher values. In those women whose menopauses had been experienced 14 to 26 years previously, the urinary titers of 17-ketosteroids were essentially normal.

The findings agree well with those of patients with intercurrent amenorrhea, presented in the previous communication. They are held to support the thesis that intercurrent or adult estrogenic ovarian failure is followed by adrenal hyperactivity. Adrenal hyperactivity under these circumstances is considered to be an expression of intimate reciprocities between the adreno-pituitary and ovario-pituitary axes, which are mediated by the pituitary. Theories, based upon these reciprocities, are applied to ovarian failure in general and to the hormonologies of the climacteric and somatic aging.

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Increased Excretion of Gonadotropic Hormone in a Pregnant Woman with Mammary Carcinoma[1]

[Mammary Carcinoma]

BERNHARD ZONDEK, M.D.

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AS HAS ALREADY BEEN REPORTED (1) patients with carcinoma excrete follicle-maturating hormone A in increased quantities. While in one liter of urine from a normal woman about 10 M.U. are found, the hormone excretion in patients with carcinoma is more than ten times higher, and on an average is 110 M.U. per liter.

The increased excretion is especially frequent in patients with malignant tumors of the genital organs. In cases of extragenital carcinomas in men, an increased hormone excretion is found only in 14.3% of the cases, while in cases of malignant tumors of the testicles of embryonal type, this increase is so frequently found that it was proposed as a diagnostic aid (2–5). In malignant tumors of the testis, besides the follicle-maturating hormone A, luteinizing hormone B, is excreted; a condition similar to that found in pregnancy. In 33.3% of women with extragenital carcinoma an increased excretion of hormone A is found as against 76.1% in carcinomas of the genital organs. It could be said that the increased hormone excretion is due to age and not to the tumor, since genital carcinoma is more frequent in women over 40 years of age and also because, after the climacterium, or after operative castration, an increased amount of follicle-maturating hormone is found in the urine (6, 7). This view is refuted by the fact that in a series of 16 women with carcinoma of the cervix occurring before climacterium, between the ages of 35 and 42, the increased excretion was found in 11 cases or 68.7% (4, 5). These findings were repeatedly confirmed (8, 9). The fact that the hormone excretion ceases with the deterioration of the general conditions of the patient, especially when moribund, confirms the view that the increased excretion is dependent on the disease (1).

The observations reported below, on a pregnant woman with a mammary carcinoma and an excessive excretion of follicle-maturating hormone, shows that the carcinomatous process is to be held responsible for the increased excretion of this hormone. It is interesting to note that in 5 cases of mammary carcinoma in nonpregnant women we were unable to find an increased excretion (1). Thus, the mammary carcinoma behaves differently during the pregnant and nonpregnant state as far as the hormones are concerned. This could be ascribed to the fact that the mammary gland is enormously enlarged and hyperemic during pregnancy.

This was a case of a 28-year-old woman, in the 8th month of pregnancy. She had a hard tumor of the breast, the size of a fist, adherent to the skin, slightly mobile, with metastatic glands in the left axilla. The woman had a radical operation[2] on Aug. 1, 1940. Six weeks later (Sept. 13, 1940) she delivered a healthy child at term. The hormonal tests were begun, unfortunately, only 3 days after the mammary-extirpation, when the patient came to our department. The urine prolan values before operation would have been, most probably, still higher. In investigations conducted in Europe (10), we found in the last months of pregnancy values of 4,000 to 12,000 M.U. of prolan A+B per liter of urine; in Palestine somewhat higher values were found, namely up to 50,000 M.U. of A+B. The prolan excretion varies greatly during the last stages of pregnancy. At times it is so low that the pregnancy reaction cannot be elicited with the usual amounts of urine (less than 400 M.U. of prolan B per

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[2] The operation was performed by Dr. E. Joseph, Head of the Surgery Department of the Rothschild Hadassah University Hospital.

liter). Shortly before delivery a maximum of 4,750 M.U. of prolan B were detected in the urine of European women (11, 12). We found much higher values here (13), up to 10,000 M.U. of A+B.

In this case of mammary carcinoma the excretion of prolan B was not increased, varying during the last 6 weeks before delivery between 1,000 to 2,000 M.U. per liter. On the other hand, we observed an excessive excretion of prolan A, 6 weeks before delivery, over 300,000 M.U. per liter. Such a high increase is never observed during the last weeks of normal pregnancy. It should be noted that the experiments were conducted on mice which are less sensitive to prolan than rats, so that 1 M.U. corresponds to 3 to 5 R.U. (4, 14). We may thus assume that on Aug. 5, 1940, at least 900,000 R.U. of prolan A per liter urine were excreted. The fact that the excretion decreased to one third of this value during 2 weeks after radical operation of the carcinoma (100,000 M.U. per liter) demonstrates that the increased production and excretion of prolan A is due to the carcinoma. The hormone content of the urine is about 10 times higher than the blood levels, that is, the organism tends, as always during pregnancy, to eliminate the hormone in the urine. After delivery the hormone values fall rapidly, so that 2 days after delivery only 1,000 M.U., and after 6 weeks less than 66 M.U. per liter are to be detected (table 1).

This case shows that in a pregnant woman with mammary carcinoma the excretion of follicle-maturating hormone was excessively high while the excretion of the luteinizing hormone oscillated within normal limits. This shows that the increased production of follicle-maturating hormone is caused by the carcinoma. The fact that only factor A has increased and not factor B is a further proof of the duality of prolan A and B.

The following tests show that we are dealing in this case with prolan and not with prosylan (from the anterior pituitary lobe): (*a*) rats are more sensitive to the hormone than mice; (*b*) the increasing hormone quantities were able to produce an increase of not more than 70 mg. in weight of the ovaries of infantile rats.

TABLE 1. PROLAN EXCRETION IN A PREGNANT WOMAN WITH MAMMARY CARCINOMA

a. Before delivery

Date	Urine		Blood	
	Prolan A	Prolan B	Prolan A	Prolan B
	M.U./liter	M.U./liter	M.U./liter	M.U./liter
5/ 8/40	>300,000	1,000		
11/ 8/40	200,000	1,500		
18/ 8/40	100,000	2,500		
30/ 8/40	100,000	1,000	10,000	1,000
13/ 9/40	delivery			

b. After delivery

15/ 9/40	1,000	1,000	neg.	neg.
27/10/40	<66	<66		

SUMMARY

In a pregnant woman with mammary carcinoma the excretion of follicle-maturating hormone A was excessively high (300,000 M.U. per liter of urine) while the excretion of luteinizing hormone B oscillated within normal limits (1,000–2,000 M.U. per liter of urine). The increased excretion of hormone A is due to the mammary carcinoma as shown by the fact that the factor A values fell rapidly after the radical operation of the carcinoma in the 8th month of pregnancy.

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Correlation of Fetal Adrenal Glands with Edema and Toxemia of Late Pregnancy

[Adrenals and Toxemia of Pregnancy]

THOMAS J. PARKS, M.D.

INASMUCH AS THE INCIDENCE of edema and toxemia of late pregnancy is reduced by diminishing the intake of the sodium ion and because of the known restricting influence of adrenal cortical hormone upon the excretion of sodium, the following investigation has been made to determine if there is any correlation between the size of the fetal adrenal glands and the incidence of edema and toxemia.

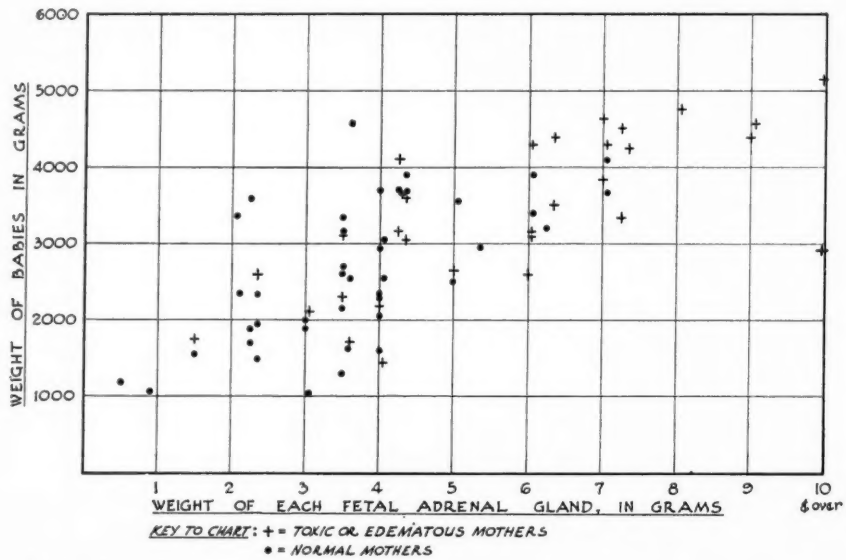

Fig. 1. CORRELATION OF FETAL ADRENAL WEIGHT WITH FETAL BODY WEIGHT IN NORMAL AND TOXIC MOTHERS.

To make this determination, the autopsy records of 131 infants who were stillborn or died in the neonatal period were studied. Of these 57 were excluded because of prematurity, insufficient data, or the adrenals were not weighed. This left 74 cases for study.

In these 74 cases the incidence of edema and toxemia in the mother was studied in relation to the weight of each fetal adrenal gland and, for comparison, to the babies' total body weight and to the weight of the liver, kidney, spleen and placenta.

COMMENTS

In considering these data, it is obvious that all the babies are, of necessity, from autopsy material. It is known that fetal mortality is high when the mother is toxic and this would tend to give one an inexact sampling of the newborn population. Also, the size of the fetuses is not constant—if the babies were of a fixed size, the adrenal glands should be bigger when the mothers are edematous. Although the big babies were found to have large adrenal glands and there was a high incidence of edema and toxemia in the mothers, it is not unlikely that there were large babies with large adrenal glands that did not die and whose mothers were not toxic.

When the babies' adrenal glands were arranged in

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groups according to weight and compared with the incidence of edema and toxemia (table 1), a curve was made extending gradually and later abruptly from 25% to 100% (fig. 1). The spleen weight (table 5) approximated this curve fairly closely. The liver weight (table 3) followed it somewhat as did the babies' total body weight (table 2). The weights of the kidney (table 4) and placenta (table 6) followed a totally different curve. The trend made by the adrenal gland was the only consistently increasing one (table 8).

TABLE 1. MOTHERS' TOXEMIA AND THE WEIGHT OF FETAL ADRENAL GLANDS

	Weight of gland in grams					Total cases
	0–2	2–4	4–6	6–8	8+	
Mothers: Edematous or toxic cases	1	6	8	12	5	32
Mothers: Normal cases	3	19	15	5	0	42
Total cases	4	25	23	17	5	74

TABLE 2. MOTHERS' TOXEMIA AND THE WEIGHT OF THE BABY

	Weight of baby in grams					Total cases
	1000–2000	2000–3000	3000–4000	4000–5000	5000–6000	
Mothers: Edematous or toxic cases	5	7	10	10	1	33
Mothers: Normal cases	10	14	15	2	0	41
Total cases	15	21	25	21	1	74

TABLE 3. MOTHERS' TOXEMIA AND THE WEIGHT OF THE LIVER

	Weight of liver in grams					Total cases
	1–100	100–150	150–200	200–250	250–300	
Mothers: Edematous or toxic cases	6	11	8	4	2	31
Mothers: Normal cases	14	21	4	3	0	42
Total cases	20	32	12	7	2	73

TABLE 4. MOTHERS' TOXEMIA AND THE WEIGHT OF THE KIDNEY

	Weight of kidney in grams					Total cases
	1–10	10–15	15–20	20–25	25–30	
Mothers: Edematous or toxic cases	7	12	8	5	0	32
Mothers: Normal cases	9	21	8	1	1	40
Total cases	16	33	16	6	1	72

TABLE 5. MOTHERS' TOXEMIA AND THE WEIGHT OF THE SPLEEN

	Weight of spleen in grams					Total cases
	0–5	5–10	10–15	15–20	20–25	
Mothers: Edematous or toxic cases	3	11	11	4	3	32
Mothers: Normal cases	9	21	10	2	0	42
Total cases	12	32	21	6	3	74

TABLE 6. MOTHERS' TOXEMIA AND THE WEIGHT OF THE PLACENTA

	Weight of placenta in grams					Total cases
	100–400	400–500	500–600	600–700	700–800	
Mothers: Edematous or toxic cases	5	3	1	0	1	10
Mothers: Normal cases	1	4	1	2	1	9
Total cases	6	7	2	2	2	19

TABLE 7. MOTHERS' TOXEMIA AND ADRENAL WEIGHT IN ANENCEPHALIC MONSTERS

No. of Cases	Average wt. of Adrenals, gm.	Edema.	Toxic.	Either T. or E.	Neither T. or E.
11	0.49	1	0	1	10

No definite correlation was found between the weight of the fetal organs and the incidence of maternal edema and toxemia. Any relationship between the weight of the fetal adrenal gland and maternal edema and toxemia is apparently of minor or secondary importance. The cause of water and sodium ion retention noted in late pregnancy cannot be explained

TABLE 8. INCIDENCE OF TOXEMIA OR EDEMA COMPARED WITH THE WEIGHT RATIO OF EACH FETAL ADRENAL GLAND PER KILO OF BABY'S TOTAL BODY WEIGHT

	Weight of each adrenal gland in gm.					Total cases
	0–2	2–4	4–6	6–8	8–	
Fetal adrenal gland in gm. per kg. baby's wt.	0.73	1.3	1.56	1.77	2.1	74
Mother's condition, toxic or edema	25%	24%	34.8%	70.6%	100%	74

by the hypertrophied fetal adrenal cortex alone according to this study. It may be that this electrolyte imbalance is in part dependent upon a deficiency of potassium ion.

The anencephalic monsters (table 7) in the entire files were studied because of the characteristic minuteness of the cortical layer of the adrenals. The weight of the adrenal glands was found to be lower than in any other group and the incidence of edema and toxemia was correspondingly less.

SUMMARY

The fetal adrenal glands and other organs have been weighed and arranged in groups according to weight. The women who gave birth to these fetuses were arranged in groups according to whether they were normal or were edematous and toxic in the last 3 months of pregnancy. An attempt was then made to see if there was any correlation between the weight of the fetal organs and the incidence of maternal edema and toxemia.

The most remarkable finding was the relative infrequency of maternal edema and toxemia in the group of anencephalic monsters.

I am indebted to Dr. B. P. Watson, Director of the Sloane Hospital for Women, for his helpful advice and permission to use this material. Also, I would like to express my appreciation for the suggestions rendered by Dr. James A. Corscaden and Dr. A. Ashley Weech and Dr. Robert F. Loeb.

Abstracts of

CURRENT CLINICAL LITERATURE

Editor: HANS O. HATERIUS. *Collaborators:* E. B. ASTWOOD, ISRAEL BRAM, JOHN C. BURCH, JOHN C. DONALDSON, MURRAY B. GORDON, ARTHUR GROLLMAN, E. C. HAMBLEN, FRANK A. HARTMAN, R. G. HOSKINS, J. E. HOWARD, ALLAN T. KENYON, J. T. LEWIS, JOSEPH M. LOONEY, A. E. MEYER, C. C. PFEIFFER, GEORGE W. THORN, EMMERICH VON HAAM.

HYPOPHYSIS

MURPHY, D. P.

The contractile response of the pregnant human uterus to posterior pituitary extract. *Am. J. Obst. & Gynec.* 41: 274. 1941.

A series of 96 women at various stages of pregnancy and labor received 375 hypodermic injections of posterior pituitary extract not exceeding 3 minims per dose (obstetrical pituitrin or pitocin). Treatments in 47% elicited no uterine responses. Positive responses occurred more commonly under the following circumstances: in multiparae rather than primiparae; during rather than before labor; late rather than early in pregnancy; after the larger rather than smaller doses; and when the uterine wall was tense rather than when it was relaxed. It was concluded that the amount of extract given and the degree of tension of uterine wall are important factors in avoiding unsatisfactory uterine responses. Small doses of the extract produce the most efficient uterine contractions.—*E.C.H.*

NIXON, N.

The treatment of Graves' disease in children. *J. Pediat.* 18: 71. 1941.

The author advises that Graves' disease be treated in children on a conservative basis. Thyroidectomy should be performed only on those children who do not show definite improvement after a comparatively long period of treatment on a carefully planned medical regime. The latter consists of complete physical and mental rest, a well balanced and high caloric diet, without restriction of proteins, and eradication of all possible sources of infection. Small doses of 1% I or 2% KI, 5 to 6 drops, reduced later to 1 to 3 drops 3 times a day, are advocated. Of 8 cases reported, 2 were treated conservatively and made a complete recovery while of the remaining 6 who were operated upon, 4 died within 1 year following operation.—*M.B.G.*

PICKER, E.

The size and number of healthy and enlarged thyroids in Danzig. *Virchows Arch. f. path. Anat. u. Physiol.* 306: 700. 1940.

While the population along the German Baltic sea coast is comparatively free of goiters, Danzig is known as a goiter region. Statistical studies on thyroids of 940 autopsy cases showed the following results: the average weight of normal thyroids does not exceed 25 g. which corresponds to the average weight of the thyroid gland in nongoitrous regions. There is a surprisingly great increase in the weight of thyroids in females during puberty (11–15 years), while there is no appreciable difference in the weight of the thyroids between the sexes before that age. There were 94 goiters in 940 cases; of those, 56 were found in persons who had lived in Danzig all the time. The highest percentage of goiter (30%) was found in women between 40–50 years of age.—*H. Smetana.*

RADWIN, L. S., J. P. MICHELSON, J. MELNICK AND S. GOTTFRIED.

Blood lipid partition in hypothyroidism of childhood. *Am. J. Dis. Child.* 60: 1120. 1940.

The serum lipid partition was studied in 54 normal children and in 17 children with either congenital or acquired hypothyroidism. The mean value in each condition was found to be as follows: Total lipids in normal children, 614 mg., in untreated hypothyroidism 1,104 mg. in treated hypothyroidism, 601 mg. Total cholesterol, in normal children, 193 mg., in untreated hypothyroidism, 353 mg., in treated hypothyroidism, 189 mg. Free cholesterol in normal children, 47.2 mg., in untreated hypothyroidism, 93.3 mg. and in treated hypothyroidism, 47.2 mg. Lipoid phosphorus in normal children, 6.7 mg., in untreated hypothyroidism, 11.0 mg. and in treated hypothyroidism, 6.3 mg., all values are in terms of mg.%. The free serum cholesterol constituted 24.5% of the total cholesterol in normal children, 27.2% in untreated and 25.1% in treated patients. The authors claim that estimation of total lipids in the serum is of greater value in the diagnosis of hypothyroidism in children than is the determination of cholesterol alone. There is no consistent relation between the total lipids value and the severity of the disease. Administration of desiccated thyroid substance to patients with hypothyroidism is followed by a reduction in the levels of all the lipid components of the blood to normal. Cessation of treatment is followed by a subsequent rise in all values.—*M.B.G.*

RASMUSSEN, H.

Influence of the thyroid hormone on heart and circulation. *Acta med. Scandinav. Supplement* 115. 1941.

Dogs were fed desiccated thyroid from 28 to 272 days in amounts ranging from 0.76 to 1.47 gm. per kg. daily in order to cause an increase in B.M.R. from 50 to 100%.

The most characteristic effect was a paroxysmal increase in heart rate up to 250 or 300 beats per minute. The systolic blood pressure showed a progressive rise but the diastolic pressure was unchanged. The arterio-venous oxygen difference was not altered but the cardiac output increased proportionally to the oxygen consumption. Death occurred from circulation failure which was accompanied by attacks of tachycardia. The essential feature of the action of thyroid on the heart rate was not an increase but a variability in the heart rate. The deleterious effect was not due to the extra work imposed on the heart but derived from the changes produced in the sinus mechanism controlling cardiac rhythm.—*J.M.L.*

Schlezinger, N. S., and W. A. Horwitz.

Neuropsychiatric disorders occurring in Cushing's syndrome. (Pituitary basophilism.) *Am. J. Psychiat.* 96: 1215. 1940.

The clinical and post-mortem findings in an unusual case of Cushing's syndrome are described. There existed in this patient a depressive psychosis and an extrapyramidal neurologic disorder, the overt development of both being associated temporally with that of the specific endocrine disorder. It is suggested that the etiology of the complex clinical picture observed in this case may be a neuroendocrine disorder on an encephalitic basis. A review of the literature reveals that mental symptoms not infrequently occur in Cushing's syndrome and that mental depression of varying degree has been a prominent feature in these psychiatric disorders. The mental symptoms may be a direct manifestation of the underlying specific endocrinopathy although the mechanism of their production remains obscure. The possibility that the form of psychiatric disorder, namely depression, in these cases bears a close relationship to manic depressive and involutional psychoses is pointed out. Patients hospitalized with depressive psychoses should be carefully examined for evidence of Cushing's syndrome.—*Author's abstract.*

Sodeman, W. A., and H. T. Engelhardt.

Renal concentration test employing use of pituitary extracts. Response of normal subjects. *Proc. Soc. Exper. Biol. & Med.* 46: 688. 1941.

Posterior pituitary extract in moderate doses produced in normal individuals an inhibition of excretion of water sufficient to raise the specific gravity of the urine to or above levels obtained with the usual test periods of water restriction.—*L. E. Gilson.*

Starr, P., and L. Davis.

Endocrine studies of patients after subtotal hypophysectomy. *Ann. Surg.* 113: 778. 1941.

A study of endocrine function in 25 patients following hypophysectomy was carried out. Complete clinical examination, including B.M.R., blood cholesterol, glucose tolerance and insulin tolerance was made. Adrenal cortical function was studied by the Wilder test and parathyroid function by determination of the blood calcium and phosphate and study of bone roentgenograms. Pituitary gonadotropic activity was estimated from the history and from the degree of sex organ atrophy. Eighteen patients with hypopituitarism and 7 with hyperpituitarism were included in the series. In the former group there were 7 patients with severe hypopituitarism with resultant total disability, more or less cerebral damage and loss of visual acuity, 4 patients with endocrine deficiency and moderate disability and 7 patients with slight endocrine deficiency and relatively little incapacity. The B.M.R. was between -30% and -40% in 4 cases and in the neighborhood of -20% in 11 cases. Blood cholesterol was significantly elevated in all 4 patients of the former group and in 6 of the latter group. Adrenal insufficiency was indicated by the results of the Wilder test in 5 of the hypopituitary patients and in 1 of the hyperpituitary group. Sugar tolerance tests revealed the expected increased tolerance in the hypopituitary patients and decreased tolerance in the hyperpituitary group. Insulin sensitivity was increased in patients with severe hypopituitarism; in the hyperpituitary group, 3 of the 7 cases were unaffected by the insulin. Sexual function and the condition of the sex organs indicated great deficiency in all of the hypopituitary cases except three. In the hyperpituitary group, 2 were normal, 2 women had amenorrhea and hot flashes and one man had impotence and atrophy similar to that seen in the hypopituitary group. The authors emphasize the following points: (1) The diagnosis of nonfunctioning pituitary or of embryonal tumors could be made earlier if patients presenting signs of loss of sexual function and secondary sex characteristics were studied with this in mind. Amenorrhea and obesity are early symptoms in women, loss of libido and diminished beard growth in men. These tumors often produce irreparable cerebral damage and extreme pituitary atrophy. If cerebral damage is great, restoration of the patient is unlikely; if pituitary function is completely destroyed, substitution therapy is not successful. (2) Endocrine studies emphasize the loss of individual pituitary functions. These may be replaced by administration of the hormones of the glands normally maintained by pituitary activity; this is especially true of the thyroid. (3) Hyperpituitarism may continue after operation and be difficult to control, or the acromegalic process may cease. A persisting disability may be due to hyperfunction of the subsidiary glands.—*Doris Phelps.*

Wilkins, L., and W. Fleischmann.

The diagnosis of hypothyroidism in childhood. J. A. M.A. 116: 2459. 1941.

The differential diagnosis of hypothyroidism in children can best be made by studies on skeletal changes in comparison with the expected change for a given age. Stunted growth, skeletal proportions (low pelvis), naso-orbital configuration, defective dental development, and epiphysial dysgenesis are the significant signs. The B.M.R. is not as reliable even when based on height rather than surface area. The decrease of cholesterol after a single dose of thyroxin gives some promise as a diagnostic procedure, since this response is not elicited in normal children or in dwarfs.—*C.P.*